Glycaemic potency of breakfast and cognitive function in adolescent schoolchildren

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TITLE PAGE

Glycaemic Potency of Breakfast and Cognitive Function in Adolescent School Children

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ABSTRACT

The aim of the present PhD was to assess how the glycaemic potency of breakfast affects cognitive function (CF) in adolescent children; using CF tests which have previously been shown to be sensitive to variations in circulating blood glucose (BG) levels; and timing the CF tests appropriately to the physiological properties of the breakfast under study. Three studies were conducted in order to test this; a cross-sectional study in children (A), a clinical study in young adults (B), and an intervention study in children (C).

(A): Sixty children aged 11-14 years were selected on the basis of being regular breakfast eaters. Their breakfast on the morning of the study was recorded. They were then categorized into four groups according to the glycaemic index (GI) and glycaemic load (GL) of the breakfast; low GI – high GL, high GI – high GL, low GI – low GL and high GI – low GL above or below the median for GI=60.6 and GL=27.5. Consumption of a low GI – high GL breakfast was associated with better performance on a majority of the tests 90-120 minutes later, suggesting a possible role for the glycaemic potency in CF.

(B): In order to test meals that were different in their GI and GL in an intervention setting, we would have to ensure that the meals selected differed in their glycaemic and insulinaemic responses. The meals were based on what the children reported eating in the cross-sectional study. Therefore, the aim of the clinical study was to measure the postprandial glycaemic and insulinaemic responses of breakfast meals differing in their GI and GL over a period of three hours after the ingestion of the meals (every 15 min during the first hour, and then ever 30 min); to measure cortisol levels over the same period; and to investigate the validity of the methods for calculating GI and GL. Ten young adults (5 males, 5 females) took part. The breakfast meals were administered in a cross-over design, and differed in their GI and GL: a low GI – high GL (1), a high – GI high GL (2a) of similar GL to (1), a high GI – high GL (2b) of similar energy and macronutrient composition to (1), a low GI – low GL (3) and a high GI – low GL (4). Insulin and glucose responses differed significantly between the high and the low GL meals, while the picture is less clear for the high and low GI meals. The iAUC (incremental area under the curve) at 120 min was predicted in a linear fashion by the calculated GL. There were no differences with regard to cortisol responses.

(C): The four breakfast meals (1, 2b, 3, 4) were administered in 74 children. The children were matched based on gender, form, age, height and BMI. Each child and their match were randomly assigned to the low or the high GL breakfast. Within each GL group, children were given high or low GI breakfasts. Mood, salivary cortisol (SC) and BG levels were measured. The low GI – high GL meal was associated with improved mood and satiating effects. When taking mood, BG and SC levels into account, the low GI meals predicted better performance on a verbal fluency task, and the high GI meals on vigilance tasks. The assumption that could be made is that the GI effects are domain specific: a low GI meal, and as a result of that lower BG levels, could result in lower activation of the HPA-axis (hypothalamic pituitary adrenal) under demanding situations (lower SC levels and feeling less 'nervous'), and therefore better declarative memory performance. Conversely, a high GI meal, and as a result of that higher BG levels, could result in stronger activation of the HPA-axis under demanding situations (higher SC levels and feeling more 'nervous'), and therefore better vigilance.

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'Τα αγαθά κόποις κτώνται.....' Ancient Greek saying

STATEMENT OF CONTRIBUTION

Tasks	Eirini Micha	Michael Nelson Supervisor	Peter Rogers	Peter Milligan	Yahya Pasdar	MSe students	UG Students	Research assistants	Lab
	Percentage of contribution								
STUDY 1									
• protocol/ study design	80	20							
• design of questionnaires/ information sheets	95	5							
• selection of CF tests	80	5	15						
• design of CF tests (mood, & task demand)	10		90						
• liaising with schools/ logistics of fieldwork	90	10							
• managing/ training researchers involved	100								
• data collection/ fieldwork	40					60			
• data coding	95	5							
• data entry	50					50			
• data cleaning	100								
• statistical design	80	20							
• statistical analysis	95	5							
STUDY 2									
• protocol/ study design	85	15							
• design of questionnaires/ information sheets	95	5							
• liaising with participants/ logistics of fieldwork	100								
• managing/ training researchers involved	100								
• data collection/ fieldwork	80						20		
• data coding	100								

CONTRIBUTORS

Tasks	Eirini Micha	Michael Nelson Supervisor	Peter Rogers	Peter Milligan	Yahya Pasdar	MSc students	UG Students	Research assistants	Lab
STUDY 2 (cont)	*Percentage of contribution*								
• data entry	100								
• data cleaning	100								
• statistical design	80	5		15					
• statistical analysis	100								
• cannulation	70				30				
• biochemistry									100
STUDY 3									
• protocol/ study design	80	20							
• design of questionnaires/ information sheets	95	5							
• liaising with schools/ logistics of fieldwork	95	5							
• managing/ training researchers involved	100								
• data collection/ fieldwork	30					25	5	40	
• data coding	100								
• data entry	30					20		50	
• data cleaning	100								
• statistical design	80	15		5					
• statistical analysis	95	5							
• biochemistry									100

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LIST OF ABBREVIATIONS

AC	Analytic class(es)
AD ACL	Activation-Deactivation Adjective Checklist
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ATP	Adenosine Triphosphate
AUC	Area under the Curve
BG	Blood Glucose
BD	Becton, Dickinson and Company
BMI	Body Mass Index
CANTAB	Cambridge Neuropsychological Test Automated Battery
CCST	California Card Sorting Test
CDC	Centers for Disease Control and Prevention
CF	Cognitive Function
CHO	Carbohydrate(s)
CNS	Central Nervous System
CRB	Criminal Records Bureau
CI	Confidence Interval
CV	Coefficient of Variation
CWT	Caroline Walker Trust
ΔR^2	Adjusted R squared
DfES	Department of Education and Skills
DH	Department of Health
DLPFC	Dorsolateral Prefrontal Cortex
DRVs	Dietary Reference Values
EDTA	Ethylenediaminetetraacetic acid
EAR	Estimated Average Requirements
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization
FBC	Full Blood Count
FSA	Food Standards Agency
g	grams
G6PDH	Glucose-6-phosphate dehydrogenase

LIST OF ABBREVIATIONS

GGE	Glycaemic Glucose Equivalent
GI	Glycaemic Index
GL	Glycaemic Load
GLUT	Glucose Transporter
GM	Grey Matter
GR	Glucose Response
Hb	Haemoglobin
HCT	Haematocrit
Ht	Height
HT	Hydroxytryptamin
HPA	Hypothalamic Pituitary Adrenal axis
ID	Iron Deficiency
IDA	Iron Deficiency Anaemia
IAUC	Incremental Area under the Curve
ICSH	International Council for Standardization in Haematology
IFCC	International Federation of Clinical Chemistry
ID	Identification number
IQ	Intelligence Quoticnt
IR	Insulinaemic Response
IVDs	*In vitro* diagnostic medical devices
KCL	King's College London
K-S	Kolmogorov-Smirnov
LIDNS	Low Income Diet and Nutrition Survey
LNAA	Large Neutral Amino Acids
m	months
MDA	Medical Devices Agency
MET	Metabolic equivalent intensity level
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
mg	milligrams
MAFF	Ministry of Agriculture, Fisheries and Food
MHRA	Medicines and Healthcare products Regulatory Agency
min	minute(s)

LIST OF ABBREVIATIONS

MRI	Magnetic Resonance Imaging
µg	micrograms
MS	Mood Scales
n	number of observations
NHF	National Heart Forum
NHS	National Health System
NDNS	National Diet and Nutrition Survey
NMES	Non-milk extrinsic sugars
NS-SEC	National Statistics Socio-Economic Classification
OFSTED	Office for Standards in Education
OUG	Occupation Unit Groups
PA	Physical Activity
PASA	Purchasing and Supply Agency
PCV	Packed cell volume
PDW	Platelet distribution width
PET	Positron Emission Tomography
PLT	Platelet count
POMS-BI	Profile of Mood States bipolar form
PQQ	Pyrroloquinoline quinine
RBC	Red blood cells
RDW	Red blood cell distribution width
RNI	Reference Nutrient Intake
SC	Social Class
SD	Standard Deviation
se	Standard error of mean
sec	second(s)
SEG	Social Economic Group
SEM	Standard Error of Mean
SES	Socio-economic status
SF	Serum Ferritin
SFT	School Food Trust
SOC	Standard Occupational Classification
SPSS	Statistical Package for the Social Sciences
STfR	Serum Transferrin Receptor

LIST OF ABBREVIATIONS

TRP	Tryptophan
TS	Transferrin Saturation
UK	United Kingdom
US	Unites States of America
VPFC	Ventral Prefrontal Cortex
WBC	White blood cells
WCST	Wisconsin Card Sorting Test
WHO	World Health Organization
WM	White Matter
Wt	Weight
y	Years

CHAPTER 1: INTRODUCTION

1.1 Outline of the thesis

This thesis is organized into six chapters.

- Chapter 1 consists of a brief introduction in the area of research.

- Chapter 2 refers to the systematic review of the subject area, providing the rationale for the study.

- Chapter 3 describes in detail the subjects and methods that were used.

- Chapter 4 presents the results of the three studies.

- Chapter 5 forms the discussion of the results that are presented in chapter 4.

1.2 Introduction

1.2.1 Focus of the thesis

Breakfast has been traditionally considered as the 'most important meal of the day'. But what is meant by this claim? Three aspects of this claim have been studied to provide support of this notion: cognitive function (CF) and academic performance; nutritional status; and body weight. Currently, the relationships between breakfast consumption and these outcomes are not clear. This thesis focuses on the relationship between the glycaemic index (GI) and glycaemic load (GL) of breakfast, CF and mood in adolescents. Academic performance is beyond the scope of this report. Nutritional status and body weight will be considered principally as possible confounders rather than as outcome variables.

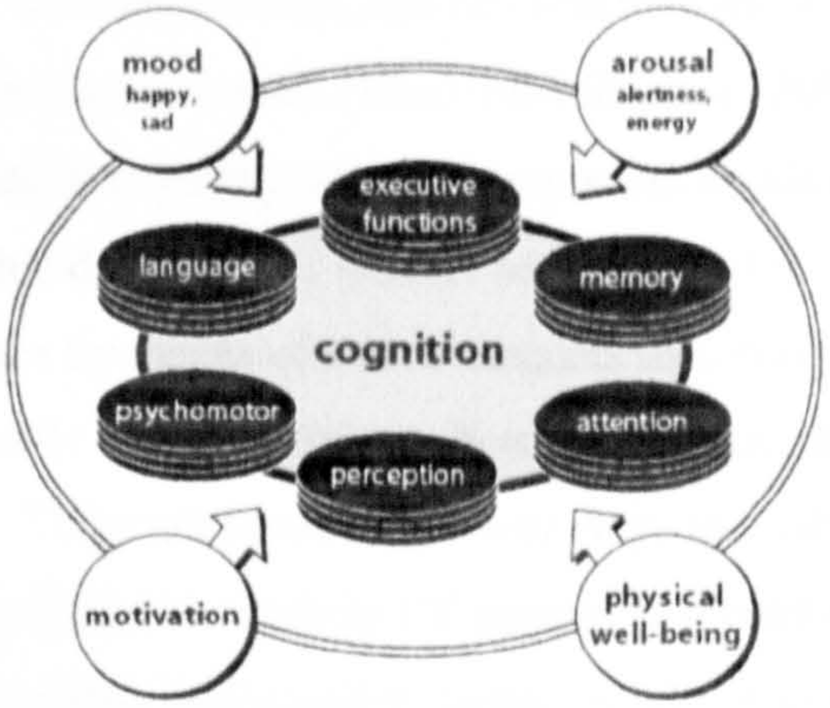
It is important for the reader to become familiar with the primary terms used in the study title that is breakfast, GI and GL, cognition, mood and adolescence.

Breakfast literally meaning 'to break the over-night fast' can be defined in several ways: the first meal after waking up; the time of the day and/ or the place that a certain meal is consumed (e.g. at home, school, work); the type of food that is actually consumed (e.g. food and/ or drink); what a person regards as breakfast; even a more rigorous definition based on a minimum number of calories consumed. A definition of breakfast is crucial to the direct comparison and evaluation of studies examining the effects of breakfast consumption on performance and overall health, and of population surveys looking into breakfast patterns. Similarly, a breakfast consumer should be defined: someone that consumes breakfast everyday, every school day, on the survey day, a minimum number of days (Rampersaud *et al.*, 2005). Nevertheless, these definitions are either lacking or are inherently different to allow for a direct comparison between studies to take place. In the current research, breakfast was defined as the first meal after waking up eaten at home or at school, including at least one food and drink (no minimum number of calories was set); a breakfast consumer was defined as someone who has breakfast at least once a week.

The issue of **GI and GL** will be addressed extensively in section 2.3 (page 55). The effects of carbohydrate (CHO) containing foods/ meals on blood glucose (BG) can be modelled by using the GI (Jenkins *et al.*, 1981) and the GL (Salmeron *et al.*, 1997). The GI reflects the speed of digestion and absorption of CHO foods and the resultant effect on the BG levels. When foods with equal amount of CHO are compared, foods with a high GI produce a higher peak in postprandial BG and a greater overall BG response during the first two hours after consumption compared with low GI foods. Furthermore, high GI foods induce a greater rise and fall in blood insulin, leading to lower concentrations of the body's two main fuels (BG and fatty acids) in the immediate post-absorptive period (Ludwig, 2002). Because GI is assessed in relation to standard quantities of CHO, it reflects the qualities of the CHO in relation to digestion and absorption and not the amount. The GL takes into account both the amount and type of CHO. It is the product of a food's GI and the total available CHO content. A low GI – high CHO food and a high GI – low CHO food will produce the same GL. However, while their effects on postprandial glucose response may be similar, the metabolic effects produced by the two foods will be very different (Barclay *et al.*, 2005). Thus, both GI and GL are best used in conjunction to describe the glycaemic potency (blood glucose raising potential) of a food or meal (ADA, 2004).

According to Drever (Drever J., 1964) **cognition/ CF** is 'a general term covering all the various modes of knowing – perceiving, remembering, imagining, conceiving, judging, reasoning'. Cognition is thus a rather broad term including various processes, which occur at different stages. Cognitive function encompasses six domains, which include memory, attention, language skills as well as perceptual, executive, and psychomotor functions, which can be further divided into sub-functions (Westenhoefer *et al.*, 2004; Schmitt *et al.*, 2005). However, it is noteworthy, that despite classification into separate domains, these various cognitive processes are interlinked and efficient functioning depends on that. Moreover, cognitive functioning can be modulated by a number of external factors, which interact with each other, such as mood, arousal (follows an inverted U-curve), individual motivation and physical wellbeing (Figure 1.1).

Figure 1.1: Cognitive functions and factors that modulate their efficiency.



Source: Schmitt (Schmitt *et al.*, 2005).

One of the main approaches of studying cognitive psychology is experimental psychology, which 'involves the use of experiments on human subjects to investigate the way in which they perceive, learn, remember or think' (Groome *et al.*, 1999). The CF tasks employed vary according to the cognitive process[1] operated to perform successfully (Carrol, 1993). Nonetheless, due to the inherent complexity of mental functions involved in any cognitive task, it is important to select CF tests that are sensitive and appropriate to the specific requirements of the study; and to time them with regard to the physiological properties of the meals under the study (Dye *et al.*, 2000; Schmitt *et al.*, 2005). When short-term nutritional interventions are employed, failure to detect an effect of a particular macronutrient could be attributed either to 'a true lack of effect, the effect of compensatory effort in experimental situations – the Hawthorne effect, or an effect on only a small component of the task (e. g., reaction time or decision time), such that an effect on overall performance is not detected' (Westenhoefer *et al.*, 2004).

[1] A cognitive process is 'any action or a series of actions in which mental contents are operated on to produce some response. These mental contents may be representations or encodings either of external stimuli or of images, knowledges, rules, and similar materials from short-term or long-term memory' (Carrol, 1993).

Mood can be defined as 'an affective state based on or closely related to emotions'; the latter are more intense, and last less than moods – seconds or minutes rather than minutes or hours (Westenhoefer *et al.*, 2004). Pubmed (MeSH term) defines mood as 'the feeling states that represent an idea or mental thought, the most direct psychic derivative of instinct and the psychic representative of the various bodily changes by means of which instincts manifest themselves'. These feeling states are usually described by words such as happy, friendly, calm, relaxed and nervous. There are potentially many ways to treat mood in studies involving dietary manipulations and/ or CF testing: as a confounder (i.e. a factor that can influence cognitive performance, initial mood); or as an endpoint of the dietary manipulation. There are many aspects that are impossible to control, such as the effect of the CF testing in stress-prone individuals, or even the mental state the participant is in the day of the testing (e.g. sleep deprived, had an argument/ fight etc). One important factor that needs to be taken into account and controlled for when assessing mood is if the participant has been exposed to the dietary manipulations before, and if it is something that he/ she habitually does; in our case how often they have breakfast and what they have for breakfast. It was recently highlighted that changes in mood are subtle, difficult to measure, disappear quickly or are the result of long-term manipulations, and therefore are not easily detected when compared to the effects on performance (Gibson & Green, 2002; Reid, 2007).

Adolescence can be defined not only as the process of physically developing from a child to an adult, but also as a critical period of major transformation of cognitive thought involving higher executive functions (e.g. abstract reasoning) (Spear, 2000). Emotional and physical changes take place, and dietary and other health-related patterns are developed; the energy requirements of this age group increase drastically making the need for meeting the energy and nutritional requirements imperative for the normal development of both parameters (Briony Thomas, 2001). However, determining the energy requirements of an adolescent can be a rather difficult procedure, because age alone can not be used as a measure of pubertal growth. The onset of adolescence differs for each individual, and adolescents of the same age often differ noticeably in size. The sexual maturity ratings (SMRs), also known as Tanner stages, are widely used to evaluate growth

and developmental age during adolescence. Nonetheless, this clinical evaluation is quite an invasive one. Therefore, in the PhD studies age, height and weight were used as indicators. The UK (United Kingdom) dietary reference values for adolescents (DRVs) (DH, 1991) divide adolescence into two age groups, 11-14 years old and 15-18 years old; the present research focuses on early adolescence.

1.2.2 Importance of breakfast and breakfast trends in school-aged populations

Breakfast has been considered as the most important meal of the day, mainly because children who have breakfast have improved nutrient profiles compared with children who skip breakfast, and as recent evidence suggests a lower Body Mass Index (BMI) as well; the latter is primarily associated with the consumption of ready-to-eat cereals. Relative to its contribution to the total daily energy intake, breakfast provides a higher percentage of micronutrients than other meals, and children who miss breakfast have lower intakes of micronutrients, especially vitamin A, vitamin B_6, vitamin D, riboflavin, folate, calcium, iron, magnesium, phosphorus, and zinc (Rampersaud *et al.*, 2005; de la Hunty & Ashwell, 2007; Affenito, 2007). Furthermore, it seems that a good quality breakfast is associated with better overall nutrient profiles and food choices compared with a low quality breakfast (Matthys *et al.*, 2007).

Research in the area of breakfast consumption habits in school children has revealed particular patterns, and predictors of breakfast consumption; breakfast skipping is highly prevalent in the United States (US) and Europe, ranging from 10-30% (Rampersaud *et al.*, 2005). It seems that adolescents are more likely to omit breakfast than any other age group and especially older adolescent girls. In the UK, a recent survey by Sodexho (Sodexho, 2005) found the following for the 8-16 age group: overall, 8% of children have nothing to eat before they go to school (5% for the boys, 10% for the girls); 7% for the 11-12 year olds, and 9% for the 13-14 year olds. In this population, overall, 86% of the participants report having a drink and 87% having food before they go to school. Among 8-16 years olds the most popular drinks include tea/ coffee (29%), fruit juice/ drink (27%) and milk (18%). Equally, the most popular food choices are cereals (64%), followed by bread/ toast (36%); only 3% report having cooked breakfast. A similar pattern of food choices was revealed by the recent National Diet and Nutrition Survey (NDNS) data in young people aged 4-18 years

(NDNS, 2000). A particular pattern that emerges from this survey is that the percentage of children not having anything to eat at school rises with age, being 12% for 15-16 year olds and 17% for girls of this age. Furthermore, the amount of money spent on the way to school on confectionery, chocolate, crisps and canned drinks has risen considerably from 2002, by 37%; the money spent is higher for boys of the same age, and rises with age. In this survey, 1,424 school children and 1,351 parents were interviewed across the UK.

In the US, the most current trend analysis of breakfast patterns in children (1-10 years) and adolescents (11-18 years) revealed a decline in breakfast consumption from 1965 to 1991 (Siega-Riz *et al.*, 1998). In preschoolers the decline was 5%; in children aged 8-10 years 9%; and in adolescents 13-20%. Older adolescent girls (15-18 years) were the age group with the greatest decline, from 88.4% in 1965 to 64.7% in 1991. The equivalent rates for adolescent boys were 89.7% and 74.9%. In 1991, the average breakfast intake for the 11-14 year old group was 80%. In this study, breakfast was defined as 'any food or beverage consumed between the hours of 05:00 and 10:00'. The sample size included 7,513 people interviewed in 1965, 12,561 between 1977-1978 and 4,289 between 1989-1991.

The observed decline in breakfast consumption was attributed mainly to behavioural changes, and secondary to the population's changing socio-demographic patterns (Siega-Riz *et al.*, 1998). Particular predictors of breakfast consumption were revealed: black adolescents were less likely than whites to consume breakfast, older adolescents (15-18 years) compared with younger adolescents (11-14 years), and female adolescents to male ones; higher parental incomes were associated with greater breakfast participation; and a one-unit increase in BMI was associated with a decreasing likelihood of having breakfast, although a specific threshold effect was not detected. Breakfast's average contribution to total daily energy intake was 21-26%, which is about one forth. The changes in breakfast food choices included: more fruit and whole grains; fewer sources of dietary fat; more low-fat milk over full-fat; and less bacon, egg, butter, margarine and white bread. However, the large percentage of adolescents that actually skip breakfast counterbalances the effect of the healthier food choices on the overall quality of breakfast. Epidemiological studies of larger

scale are needed in the UK, where similar parameters will be studied, and perhaps similar trends will be revealed.

But what should the macronutrient composition of breakfast be? The estimated average requirements (EAR)[2] of energy for males and females aged 11-14 years old are 2,200 and 1,800 kcal/day, respectively (DH, 1991). The nutrient-based standards by the Caroline Walker Trust (CWT) and the National Heart Forum (NHF) suggested that breakfast should cover 20% of the EAR, and the intake of the macronutrients should be 20% of food energy for total CHO and fat, and 20% of the Reference Nutrient Intake (RNI)[3] for protein (CWT & NHF, 2005). Recently, the Government introduced new food-based standards, the legislation for which came into effect in September 2007. The School Food Trust (SFT) has published a guide to introduce these food-based standards, for both lunch, and foods other than lunch (SFT, 2006a; SFT, 2006b).

1.2.3 Breakfast, Cognitive Function and Mood

The potential effects of nutritional factors on CF and behaviour are of significant importance, given that childhood and especially adolescence are crucial periods for the development and maturation of both body and mind. The physical growth is accompanied by the acquisition of factual knowledge, behavioural and emotional skills, which help the child cope with the everyday demands of our modern society.

The common belief that skipping breakfast can be disadvantageous to performance has been greatly influenced by some early studies looking into the effects of skipping breakfast *vs.* consuming breakfast on physiologic responses (Tuttle *et al.*, 1950; Tuttle *et al.*, 1953; Tuttle *et al.*, 1954). These early studies, which are jointly known as the Iowa Breakfast studies, have been criticized to a great extend for their small subject numbers, the use of choice reaction time as the only measure of performance, as well as the use of subjective measures of assessment (e.g. records of teachers with regard

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[2] The EAR for energy or a nutrient is the amount, which any stated group of people will need, on average. Half of the population will have needs greater than this, and half will have needs below this amount (DH, 1991).
[3] The RNI is the amount of a nutrient, which is sufficient to meet the dietary requirements of nearly all in the group (97%). By definition, many within the group will need less (DH, 1991).

to the change in the attitudes and the scholastic attainments of the students) (Dickie & Bender, 1982). Nonetheless, these same studies constituted the basis for future research in examining the effects of breakfast omission on cognitive and academic performance in school children.

Since then, a number of studies have examined the effects of breakfast omission on the CF of school children. Breakfast consumption has been associated with improved school attendance and better academic performance at school. Omission of breakfast may interfere with cognition and learning. The importance of breakfast consumption for scholastic achievement is illustrated in the effects of breakfast on CF. The cognitive functions that seem to be adversely affected by the omission of breakfast are mainly mnemonic processes (Smith *et al.*, 1999; Wesnes *et al.*, 2003; Mahoney *et al.*, 2005), problem solving tasks (Pollitt *et al.*, 1981; Pollitt *et al.*, 1982), arithmetic (i.e. computational skills) and continuous performance tasks (Conners & Blouin, 1982). Sustained attention seems to be less vulnerable to the effects of breakfast omission (Pollitt & Mathews, 1998; Smith *et al.*, 1999; Mahoney *et al.*, 2005). Mood has not always been accounted for, but breakfast administration has been reported either to have a positive effect (Wesnes *et al.*, 2003) or a no effect (Mahoney *et al.*, 2005). Regardless of breakfast's beneficial effects, many children go to school without breakfast, and skip breakfast more often than any other meal (Nicklas *et al.*, 2004).

Despite the wealth of studies that have been conducted in this area, the relationships between CF and breakfast consumption in school children are not clear. This is due in part to variations in design and failure to account for the possible confounding factors that could affect CF. These confounding factors include: iron deficiency (ID) and iron deficiency anaemia (IDA) (McCann & Ames, 2007); overall nutritional status (Dye *et al.*, 2000); variation from habitual breakfast eating habits (Kanarek, 1997; Lopez-Sobaler *et al.*, 2003); the type of breakfast itself, which may vary in terms of composition, energy load, time of consumption (Dye *et al.*, 2000; Gibson, 2007), and the subsequent timing and selection of the appropriate CF tests (Schmitt *et al.*, 2005); inter-individual differences and underlying physiological adaptations, such as behavioural arousal, individual effort/ motivation, and mood (Dolan, 2002; Blatter & Cajochen, 2007); socio-economic status (SES) (Bradley & Corwyn, 2002; Power *et al.*, 2006); and glucose tolerance (Gibson, 2007). These limitations clearly support the

need for well-designed and well-controlled intervention studies, where the role of breakfast on CF and mood will be elucidated.

At the very least, the studies thus far have provided proof in support of the assumption that the brain is vulnerable to the effects of brief fasting (i.e. overnight fast). However, the macronutrient composition of breakfast that could best facilitate performance after an overnight fast remains unclear. As glucose is the major source of energy for the central nervous system (CNS), it has been recently suggested that glucose could be mediating the memory enhancing effect of breakfast. The effects of glucose on CF follow a narrow inverted-U dose-response curve, with particular effect on mnemonic processes, and tend to selectively enhance performance when participants are engaged in sufficiently demanding cognitive activity (Gibson, 2007). It has been suggested by Gibson (Gibson, 2007) that an interaction between glucoregulatory processes, arousal and cortisol secretion is the underlying mechanism mediating the glucose effect on CF and mood. Therefore, the effects are expected to be highly variable. The effects of BG levels on subsequent mood have received little attention, and especially in the context of a composite breakfast meal. The application of an increased cognitive demand seems to be an important factor in the association between falling BG levels and falling levels of subjective energy. Time is also a major variable when examining the effects of different glycaemic CHO[4] on mood. It could be argued that when consuming a glucose load (e.g. a drink) or a sugary snack a short-term increase in energy would be expected after 15, 30, or 60 minutes; whereas this would be followed by a long-term fall in subjective energy and mood – usually detectable after 2 hours (Benton & Nabb, 2003). The opposite could be expected for a low GI food or meal.

The interest nowadays is in low GI meals, and whether these could facilitate performance and mood in the hours that follow ingestion by minimizing glycaemia fluctuations (Benton *et al.*, 2003; Mahoney *et al.*, 2005; Nabb & Benton, 2006; Ingwersen *et al.*, 2007; Gibson, 2007). These studies support the hypothesis that a low GI meal has beneficial effects on CF one to two hours after the start of breakfast, when the BG levels have returned to baseline, though the mechanisms are not

[4] According to FAO, a more appropriate substitute for the terms 'available' and 'unavailable' today would be to describe carbohydrates as either glycaemic (providing energy for metabolism) or non-glycaemic (FAO/WHO, 1998).

elucidated. It could be that high GI meals are associated with increased glucose levels and therefore cortisol secretion under stress (i.e. demanding tasks); effects which seem to favor vigilance, but impair memory. Therefore, it is important when evaluating the effects of GI on CF to control for GL, to include a selection of tests that assess both mnemonic processes as well as vigilance, to time these appropriately, and to measure both glucose and cortisol levels.

The previous summary makes clear that the role of GI, and/ or GL on CF has not yet been clearly demonstrated or elucidated. The main purpose of the PhD research, therefore, was to show that the GI and/ or GL of breakfast can influence cognitive performance and mood, when all major confounders have been properly controlled for.

CHAPTER 2: LITERATURE REVIEW

2.1 Nutritional influences of Breakfast on Cognitive Function

2.1.1 Summary

The brain is the most metabolically active part of the human body; while it contributes only 2% to the average person's body weight, it accounts for approximately 20% of the basic metabolic rate (Benton, 2001). The major source of energy for the brain is glucose, which is essential for the normal functioning of the CNS. Adequate brain function is vital for efficient CF and organized behaviour, as well as for the performance of many voluntary and involuntary functions. A balanced diet that meets the needs in all macro and micronutrients seems to be vital for the maintenance of brain function; chronic malnutrition, and as a result of that deficiencies in a number of vitamins and minerals impede cognitive performance and development, as well as affecting behaviour adversely (Wachs, 1995; Kretchmer *et al.*, 1996; Bryan *et al.*, 2004; Fanjiang & Kleinman, 2007). The question that arises is whether short-term changes in the available substrates of an individual can exert an effect on neural functions, such as memory, learning, information processing, and mood. These short-term changes may be the result of skipping a meal or eating a meal of specific composition after an overnight fast. The present review will try to answer the previous question by examining whether skipping breakfast, and mainly whether eating a breakfast of specific composition, can affect the cognition and mood of school-aged children. The long-term studies, which evaluate the effects of school feeding programmes on the scholastic achievement and the nutritional status of school-aged children, are beyond the scope of the present review (Pollitt & Mathews, 1998).

The interest in the effects of skipping breakfast on CF in school-aged populations (especially the 9-11 year old group) has been based on the greater metabolic stress imposed by brief fasting on children (Pollitt *et al.*, 1981), their increased energy requirements, and breakfast's substantial contribution to daily energy and nutrient requirements (Rampersaud *et al.*, 2005). These parameters have provided the theoretical background for the hypothesis that omitting breakfast might have a detrimental effect on cognitive performance. However, not all studies undertaken have supported this hypothesis. This can be attributed to methodological problems, in

addition to not taking into account all possible confounding factors. These, as well as other issues will be discussed in this chapter.

2.1.2 Breakfast *vs.* no breakfast

Studies comparing the effects of breakfast omission on CF have mainly focused on the 9-11 year old group; only a limited number of studies have focused on adolescents (Dickie & Bender, 1982; Cromer *et al.*, 1990; Vaisman *et al.*, 1996; Wesnes *et al.*, 2003). Furthermore, the primary focus of these studies has been cognition, and not mood. In comparison to earlier studies, recent ones have been more carefully designed: cross-over designs where all participants are exposed to all experimental conditions have been used; and appropriate CF tests (i.e. sensitive) to the experimental protocol have been administered.

The plethora of studies looking into the effects of skipping breakfast on CF are in support of the hypothesis that skipping breakfast has an adverse effect on cognition (Pollitt *et al.*, 1981; Pollitt *et al.*, 1982; Conners & Blouin, 1982; Simeon & Grantham-McGregor, 1989; Chandler *et al.*, 1995; Vaisman *et al.*, 1996; Benton & Parker, 1998; Smith *et al.*, 1999; Wesnes *et al.*, 2003; Mahoney *et al.*, 2005). This is why the Proceedings of the International Symposium on Breakfast and Performance in Napa concluded that 'at the very least, breakfast consumption improves school attendance and enhances the quality of a student's life', while the mechanisms that are responsible for this effect remained to be elucidated; nutritionally at-risk children could be particularly benefited (Pollitt & Mathews, 1998).

The CF tests that have been widely used to evaluate the effects of the breakfast *vs.* the no breakfast condition are the ones assessing several domains of memory. Benefits have been reported regarding short-term memory (Pollitt *et al.*, 1981; Simeon & Grantham-McGregor, 1989; Vaisman *et al.*, 1996; Mahoney *et al.*, 2005), episodic memory (Wesnes *et al.*, 2003), spatial memory (Smith *et al.*, 1999), problem solving tasks (Pollitt *et al.*, 1981; Pollitt *et al.*, 1982), verbal fluency tests (Simeon & Grantham-McGregor, 1989; Chandler *et al.*, 1995), arithmetic (i.e. computational skills) and continuous performance tasks (Conners & Blouin, 1982), word and paragraph recall (Benton & Parker, 1998). Attention seems to be vulnerable to the

effects of skipping breakfast (Wesnes *et al.*, 2003). However, the reported data do not allow a direct comparison, since different attention tasks have been employed, and the data are far from being conclusive. Overall, the data seem to be less supportive of an effect of breakfast omission on this cognitive domain, and especially sustained attention (Simeon & Grantham-McGregor, 1989; Smith *et al.*, 1994; Pollitt & Mathews, 1998; Smith *et al.*, 1999; Mahoney *et al.*, 2005).

On the contrary, there are some studies that have not yielded any statistically significant effects between the breakfast *vs.* the no breakfast condition, even when aspects of CF that have been shown to be selectively affected by breakfast omission were assessed, including memory, attention, computational skills and continuous performance tasks (Dickie & Bender, 1982; Cromer *et al.*, 1990; Lopez *et al.*, 1993; Lloyd *et al.*, 1996; Green *et al.*, 1997). Such a lack of effect could be attributed to different methodological designs and to not controlling for potentially confounding factors (see section 2.1.4, page 35), including different meal composition between studies, and timing of the CF tests in relation to the physiological properties of the meals under study. This makes the comparison among these studies even more difficult.

Studies looking into the effects of breakfast omission have revealed that individual differences in task engagement/ motivation, and arousal or stress may complicate our understanding of the effects of breakfast, or of the breakfast-dependent changes in BG, on cognitive performance. This is why there is a need for more tightly controlled studies in larger samples of children, where possible confounding factors should be accounted for. Nonetheless, despite the conflicting results, studies looking into the effects of breakfast omission on CF have at the very least provided evidence in support of the assumption that the brain can no longer be considered insensitive to short-term fasting. The effects of breakfast administration *vs.* no breakfast on mood are similarly conflicting as both an improvement (Wesnes *et al.*, 2003) and a no effect (Mahoney *et al.*, 2005) have been reported.

2.1.3 Macronutrient composition of breakfast

Differences in breakfast composition may partially account for the observed inconsistencies of the effects of breakfast consumption on CF. The characteristics of the meal itself, such as its composition, size, time of consumption, and subsequent timing and selection of the CF tests, may influence cognition and mood by inducing metabolic alterations, such as changes in the levels of blood glucose, insulin and cortisol, and the concentration of neurotransmitters (Kanarek, 1997; Pollitt & Mathews, 1998; Dye *et al.*, 2000; Gibson, 2007).

Furthermore, when evaluating the effects of breakfast meals on CF, their differences in content, volume, osmolarity and oro-sensory properties (such as taste, pleasantness) should always be considered, as these characteristics may exert various effects on behaviour and CF of different individuals; for example increased volume might affect gastric emptying and feelings of discomfort (Schmitt *et al.*, 2005). So, at the very least, when different breakfast meals are assessed, they should have the same total volume. Nonetheless, most studies until recently (Wesnes *et al.*, 2003; Nabb & Benton, 2006; Ingwersen *et al.*, 2007) have not tried to separate or control for the effects of different breakfast meals from those of breakfast timing, and subsequent testing, on CF (Pollitt & Mathews, 1998). Furthermore, breakfast has been mainly described by its energy content as high, low or no energy, and not by its macronutrient content (protein, fat, CHO) or glycaemic potency.

When the energy load is considered, Michaud (Michaud *et al.*, 1991) reported that a high energy breakfast providing 25% of the average daily energy requirements in late adolescents resulted in improvements in short-term memory, but an adverse effect on concentration, three hours later, in comparison to a low energy breakfast (less than 10% of daily energy requirements). A similar evaluation in 10 year old children reported an improvement on a creativity test (Wyon *et al.*, 1997). In a recent study by Nabb and Benton (Nabb & Benton, 2006) young adults having eaten breakfast meals of less than 150kcal recalled more words in a word recall task than those having eaten 251-280kcal; information processing and simple reaction times, on the contrary, were unaffected by the caloric content of the breakfast meals. Besides, as highlighted by Gibson and Green (Gibson & Green, 2002), meal composition may be more

influential than energy *per se*. Mood appears to be particularly unaffected by alterations in meal size, unless very little is eaten or stress is present. Michaud (Michaud *et al.*, 1991) found that alertness and tranquility were not affected three hours later by a large *vs.* a small breakfast in adolescents. Wyon (Wyon *et al.*, 1997) observed that participants reported feeling bad and more hungry when very little was eaten for breakfast. As far as stress is concerned, it seems that higher energy intake can actually prevent deterioration of mood when subjects are stressed by noise (Macht, 1996). Recently, the effects of breakfast size on behaviour in children were studied (Benton & Jarvis, 2007). Pupils who had consumed a low energy breakfast (<150kcal) were less engaged and more distracted than those who had eaten more.

When the actual macronutrient composition of breakfast is considered, a low fat (27% of energy), high CHO (62% of energy) breakfast resulted in a reduction in subjective fatigue in comparison to a medium fat (44% of energy), medium CHO (47% of energy), and a high fat (56% of energy), low CHO (34% of energy) iso-energetic breakfast (Lloyd *et al.*, 1996). Similarly, a high fibre (19.1g), CHO rich (61% of energy) meal produced improved subjective alertness rating and was more filling compared with a low fibre (1g), CHO rich (71% of energy) or a fat rich breakfast (~50% of energy) (Holt *et al.*, 1999). Consumption of a high CHO (71% of energy), complex CHO meal was associated with lower levels of fatigue and higher levels of satiety compared with the consumption of a high CHO (74% of energy), simple CHO meal of similar macronutrient composition (Pasman *et al.*, 2003). Gibson and Green (Gibson & Green, 2002) concluded that in general, fatigue seems to increase and alertness to decrease after the consumption of a high fat meal compared with a low fat – high CHO meal, when protein is kept constant. Recently, it was highlighted that fibre might evoke a higher satiety effect when compared with fat or rapidly digestible CHO (Slavin & Green, 2007). On the contrary, it was found that mood improved in the group of subjects that received a cooked breakfast of eggs and bacon in comparison to the group that received toast and cereal (Smith *et al.*, 1994). The high fat breakfast resulted in subjects being less discontented and more sociable. Nonetheless, many factors could have attributed to that, such as that the individuals might have linked the cooked breakfast with past experiences, for instance a sociable occasion, or a comfort food.

The effect of a high energy, and/or a high fibre – CHO rich meal on subsequent cognitive performance and mood might be mediated by their effects on BG levels and brain neurotransmitter synthesis. There are many studies showing that glucose administration can result in an improvement in cognition (see section 2.2.2, page 42). The influences of altering the content of all three macronutrients on CF, but not mood has recently been assessed by Nabb and Benton (Nabb & Benton, 2006) in a factorial design. Memory was better when low levels of protein were consumed (1.7g), and after a combination of low CHO (24g) and fat (1g) intake; both dietary manipulations were associated with small increases in BG levels. On the contrary, a high GL meal (GL=53) was associated with more 'correct' responses on a reaction task. The issue of GL and GI is dealt with in detail in section 2.3.3 (page 60).

The effects of different CHO to protein ratios in breakfast on CF and mood were tested in young adults (Fischer *et al.*, 2002). They found that apart from a short positive effect of the medium to high GI CHO-rich meal (4:1) on attention, overall performance was better after the balanced (1:1) and the high protein meal (4:1). The effect of the two latter meals was attributed to a decreased variation in the glucose metabolism, and/ or increased metabolic activation and neurotransmitter synthesis. The researchers concluded though that needs to be investigated is if 'this also holds for a low GI CHO-rich meal'. A particularly interesting finding by Markus (Markus *et al.*, 1998; Markus *et al.*, 1999) was that the induced increase in blood tryptophan (TRP) levels by a high CHO very low protein meal seems to protect stress-prone individuals from a deterioration in mood by producing serotonin under challenge.

2.1.4 Potential confounding factors

Despite the wealth of studies that have been conducted in this area, the relationships between CF and breakfast consumption in school children are not clear. This is due in part to variations in design and failure to account for possible confounding factors that could affect CF. Extra care was taken to ensure that all these factors were accounted for and/ or measured in the present PhD project. These confounding factors include: iron deficiency (ID) and iron deficiency anaemia (IDA); socio-demographic characteristics; inter-individual differences; the type of breakfast itself (see section 2.1.3, page 33), which may vary in terms of composition, size, time of consumption,

and the subsequent timing and selection of the appropriate CF tests; and lifestyle factors. Each one of these potentially confounding factors is briefly mentioned here, along with important relevant reviews.

Iron status has been linked to cognitive performance and behaviour (McCann & Ames, 2007). Existing data suggest that iron deficiency, even when it is not severe enough to cause anaemia (IDA), is a risk factor for poor educational performance in children (Gordon, 2003) and adolescents (Nelson *et al.*, 1994; Nelson, 1996; Halterman *et al.*, 2001). Iron supplementation seems to reverse these effects on arousal, attention span, memory and concentration (Sachdev *et al.*, 2005). The synergistic effect of IDA in combination with other forms of malnutrition and other risk factors may affect educational performance more strongly. The detrimental effects of iron deficiency seem to be more profound in adolescent girls, where the onset of menstruation, and dietary habits such as vegetarianism and dieting to lose weight, contribute to an increased risk (Nelson *et al.*, 2001).

Socio-demographic characteristics such as socio-economic status (SES), gender and overall nutritional status have been linked to performance. Socio-economic status is another important confounding factor that has not always been accounted for, and seems to predict scholastic achievement in school-aged children; children from lower SES appear to have poorer cognitive performance. Specifically, cognitive and academic performance has been linked to a variety of socio-economic indicators; family income and parental education can be used as predictors of academic performance (Bradley & Corwyn, 2002; Arnold & Doctoroff, 2003; Power *et al.*, 2006). Gender also needs to be accounted for, as there seems to be a hormonally influenced sex difference, favouring pre-school girls, for processing and interpreting non verbal behaviour, facial expressions and language comprehension and production (Geary, 2006), and boys for arithmetic cognition (Carr & Davis, 2001; Rocha *et al.*, 2005). The detrimental effects of breakfast omission on cognitive and academic performance appear to be more profound in nutritionally at-risk children, effect which is independent of Intelligence Quotient (IQ) (Simeon & Grantham-McGregor, 1989; Chandler *et al.*, 1995; Pollitt *et al.*, 1996). Poor nutrition (both quantity and quality) has been proven to be associated with poorer performance, and as such malnourished children are more likely to perform worse compared with the well-nourished ones

(Stevenson, 2006). Interestingly enough, well-nourished children were not affected by the omission of breakfast in either three studies (Simeon & Grantham-McGregor, 1989; Chandler *et al.*, 1995; Pollitt *et al.*, 1996). These findings in Jamaica and Peru are in contrast with the ones in the United States (Pollitt *et al.*, 1982; Conners & Blouin, 1982), where breakfast omission affected well-nourished children as well. It has been suggested that the reason for this inconsistency could be that in developing countries, even adequately nourished children are more used to missing breakfast compared with their US counterparts; and as such less vulnerable to its detrimental effects (Pollitt *et al.*, 1996).

Inter-individual differences could explain much of the variability in the results (Blatter & Cajochen, 2007). These differences include individual variability in arousal (Pollitt *et al.*, 1981; Westenhoefer *et al.*, 2004), individual effort/ motivation, anxiety/ stress, perceived difficulty, baseline mental state/ mood (e.g. fatigue, hunger, thirst, effect of testing) (Dolan, 2002). All these can vary under experimental situations and task demand, and could influence the outcome of dietary manipulations on performance. Other factors include the hours of sleep and the quality of sleep, as sleeping less and sleeping disturbances have been associated with decreased concentration and increased basal cortisol levels (Capaldi, V *et al.*, 2005; Blatter & Cajochen, 2007); and time since awakening, as earlier risers may have both lower BG levels and less 'sleep inertia' (Gibson & Green, 2002). The length of overnight fast and glycogen stores at the time of participation in the study could also influence glycaemic responses the morning after (Pollitt *et al.*, 1981; Brouns *et al.*, 2005). A limited number of studies have ensured that glycogen stores are not depleted by providing a standardized meal the evening before the study, or by at least controlling for it (Dye *et al.*, 2000). It has been suggested that a profile of good glucose tolerance could be associated with enhanced performance on the cognitive tasks (see 2.2.2, page 42). It would be interesting to examine whether improvements in glucose tolerance induced by the type of breakfast being consumed would be associated with improved cognitive functioning. Hydration is another factor that has been linked to performance in young adults; mild dehydration (as little as 2% of loss of body weight as water) has been linked to impairments in performance and decreased concentration (D'Anci *et al.*, 2006). Furthermore, it is imperative that these studies follow highly controlled protocols with regard to the timing of the CF testing and any physiological

measurements, to avoid any potential circadian influences on performance (Blatter & Cajochen, 2007). For example, cortisol, a steroid hormone and the by-product of the Hypothalamic Pituitary Adrenal (HPA)-axis, has its highest levels in the morning (highest after waking up) and drops throughout the day (Capaldi, V *et al.*, 2005). Even environmental factors (e.g. noise, ambient temperature, lighting) can affect within-subject variability; hence, standardisation of these factors is necessary (i.e. using secluded rooms) (Schmitt *et al.*, 2005).

Moreover, the **habitual breakfast eating habits** of the participants should be accounted for, as deviation from normal breakfast (macronutrient composition, or no breakfast at all) can impede performance, and daily consumption of breakfast has been associated with improved response to stress and lower cortisol levels (Lloyd *et al.*, 1996; Kanarek, 1997; Smith, 2002; Lopez-Sobaler *et al.*, 2003). Regular breakfast consumption seems to improve overall nutritional status, as well as subsequent mood, and provides students with energy (Lombard, 2000). It appears that the diet to which the individual is best adapted produces a 'neurochemical response or metabolic activation' (for example, changes in serotonin or glucose), and that cognitive performance seems best after ingestion of the macronutrient composition closest to the one habitually consumed, when short-term effects are being investigated (Dye *et al.*, 2000).

In females, the **menstrual cycle phase** may also exert changes in mood, and subsequently performance (Rogers *et al.*, 1992; Davydov *et al.*, 2005). There is great variability in mood changes associated with the premenstrual phase; negative moods have been reported, such as tension and anxiety, irritability and depression, as well as improved mood. It seems that the follicular and luteal phases along with the hormones associated with these, do not determine positive or negative moods *per se*, but rather interact with arousal related factors (e.g. environmental stress) to intensify or reduce emotions.

Dieting to lose weight is another parameter that might influence cognition and mood, especially in women. Females who are on a diet restriction programme, appear to be vulnerable to cognitive impairment, due to reduced sustained attention and working memory capacity (Gibson & Green, 2002). The effects of dieting on performance

appear to be psychological rather than physiological, as impairments in performance are detectable even when the dieters have not lost any weight, and are more profound to those people that have been dicting for the shortest period of time (Green & Rogers, 1995). In females, it is also known to influence serotonin-mediated neural responses, and due to that, mood (Dye *et al.*, 2000).

The cognitive function tests selected may not always be appropriate for the group being studied (Pollitt & Mathews, 1998; Schmitt *et al.*, 2005; Gibson, 2007). The particular characteristics of the group under study should always be accounted for (e.g. age, previous exposure to such testing, either paper or computer based). In selecting a CF test, much depends on 'whether an immediate (short-term) effect of food is expected or whether a chronic, long-term adaptation to a diet is being examined' (Dye *et al.*, 2000). For example, when the effects of glucose are examined, it has been shown that more demanding tasks are more sensitive to a facilitative effect of glucose; the duration of the task is of great importance, and also the cognitive domain (e.g. memory, vigilance) (Gibson, 2007).

Lifestyle factors, such as caffeine and alcohol consumption, and smoking. Caffeine has long been considered as a 'psychostimulant', for both mood and cognitive performance. Nonetheless, recent evidence suggests that the benefit gained from regular caffeine consumption might be little or even none; but rather regular caffeine consumption causes dependency, and as such any observed deleterious effects on mood and performance might be due to caffeine withdrawal (James & Rogers, 2005; Rogers, 2007). Alcohol affects CF, and especially learning and memory (i.e. worse performance); younger people appear to be more sensitive to the effects of alcohol (White & Swartzwelder, 2004). To what extent, that depends whether it is consumed on its own or as part of a meal (less profound effect). In general, it seems to affect performance negatively on a number of tasks, such as psychomotor tracking, driving tasks, perception, sustained attention, and information processing (Dye *et al.*, 2000). Furthermore, smoking has been linked to cognitive performance. Recent evidence suggests that adolescent regular tobacco smokers have impaired cognitive performance, and that cessation of smoking brings about detrimental effects on verbal and working memory capacity (Jacobsen *et al.*, 2005).

2.2 Cognitive Function and Glucose

2.2.1 Neuronal metabolism and Glucose

The brain is considered the most important organ of the human body, as it controls the involuntary and conscious actions of the organism (see Appendices I.1 and I.2 for information regarding the structure and functions of the brain, as well as the adolescent brain, respectively). It relies on a constant supply of nutrients from blood for its optimal functioning through the blood-brain barrier, as it can not store oxygen and has limited storage of glycogen. The blood-brain barrier has a lipophilic nature obstructing hydrophilic substances like ions (e.g. bicarbonate, hydrogen) from crossing. Nonetheless, non-lipophilic substances like glucose, and other substrates that are important for cerebral metabolism (e.g. lactate, aminoacids), can enter the brain by a mechanism of facilitated diffusion. Glucose enters the brain (blood-brain barrier) by binding to a facilitative transport protein (glucose transporter 1 (GLUT1); astrocytes and oligodendrocytes express GLUT1; microglia express primarily GLUT5; and neurons express GLUT3 (Paulson, 2002).

Glucose is the major fuel of the brain under both resting and functioning conditions, as it is readily available in the systematic circulation in normally regulated levels, and can also be rapidly transported to the cerebrum through the blood-brain barrier. The brain takes up only about 10% of glucose in the blood, and any excess glucose is transported out of the brain and back into the venous system (Brown & Ransom, 2007). The extracellular glucose concentration in the brain and the cerebrospinal fluid is about 40% of the concentration in the blood. If the need for glucose is increased (e.g. under intensive functioning) then the transport back to the blood decreases, and therefore the extracellular glucose concentration in the brain. This could potentially lead to insufficient glucose supply to all parts of the brain (Paulson, 2002).

It has been recently found that under resting conditions astrocytes and neurons have similar oxidative capabilities to glucose; oxidative metabolism in cortical glial cells or astrocytes accounts for ~30% of total tissue oxygen consumption in the brain cortex, which is analogous or even higher to the astrocytes' tissue volume (20-25%). Nonetheless, only astrocytes are capable of storing glucose as glycogen, which under

brain activation has a high turnover (increased glycogenolysis). Brain glycogen is intended for local use only. Glycogen seems to be highest in brain areas with the greatest synaptic density, like the hippocampus and the cerebral cortex (grey matter structures), reflecting the increased energy required in synaptic transmission (Hertz *et al.*, 2007).

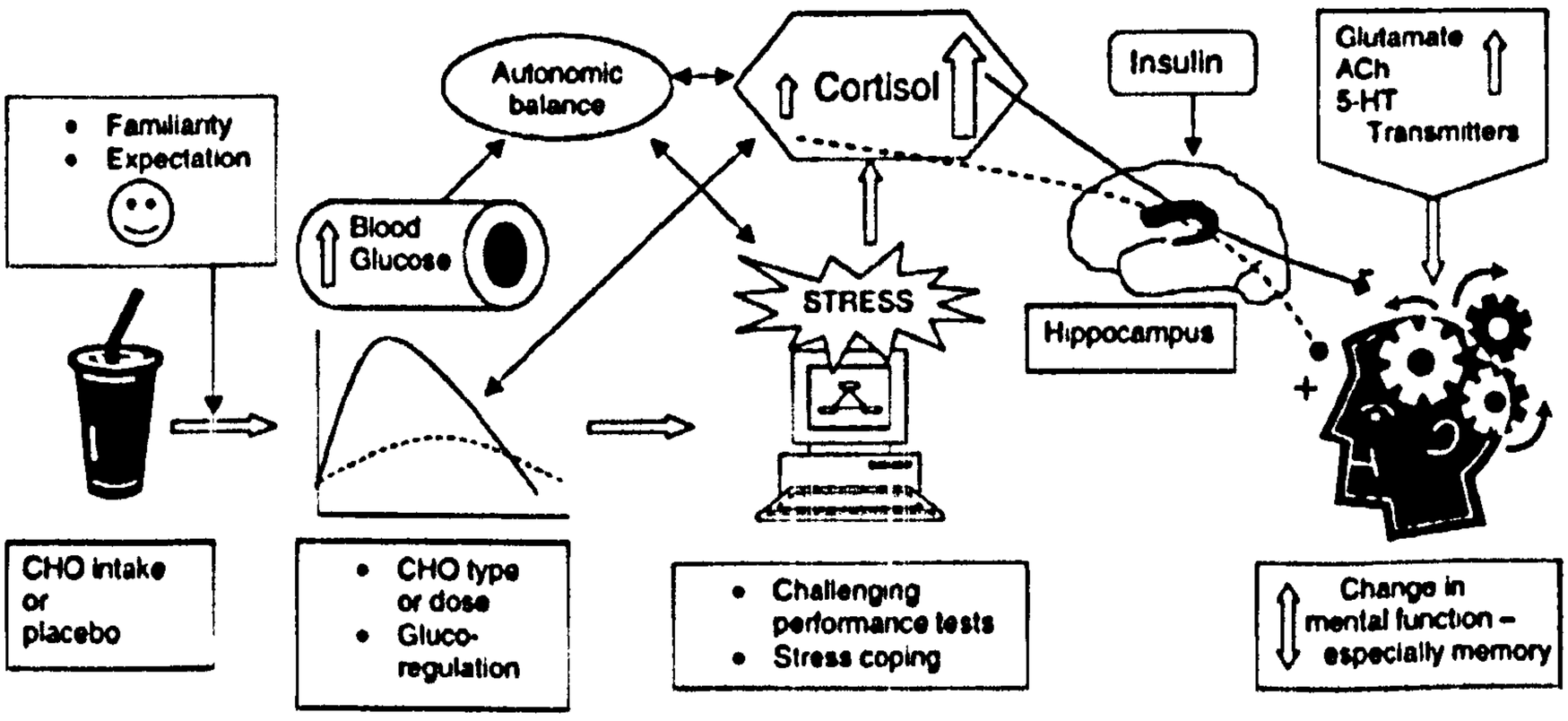
Under normal conditions the ratio between oxygen (oxidative metabolism, CMR_{O2}) and glucose consumption (cerebral glucose utilization, CMR_{glc}) is six, as essentially all glucose is metabolised into water and carbon dioxide ($C_6H_{12}O_6+6O_2 \rightarrow 6CO_2+6H_2O$). During activation, glucose consumption increases in excess of oxygen consumption reflecting non-oxidative metabolism, and this ratio drops well below six. Brain tissue consumes about 100μmoles of oxygen per g of tissue per hour implying complete oxidative metabolism would consume 600μmoles glucose/g/h. Since brain glycogen concentration is between 5 and 15pmoles/μg protein (~0.5-1.5μmoles/g tissue), all brain glycogen would be consumed within a few minutes, if glucose was not available. This is why the brain relies on a constant supply of glucose. From the time glucose enters the blood, it takes over six hours to be stored as glycogen. The role of glycogen is to provide energy substrate under hypoglycaemic conditions when the rate of glucose delivery from the blood is insufficient to meet immediate energy requirements (prolong neuronal survival), and during increased brain activation when glucose in the brain is insufficient to meet the increased energy demand. Under hypoglycaemia glycogen can preserve brain function for up to 90 min, contradicting the notion that glycogen stores in the brain are too small to support brain function. During functional activity, increased blood flow provides the working area with the necessary oxygen and glucose. Astrocytic glycogen is broken down to lactate, which is more rapidly available than the delivery of glucose, and can provide more quickly the area in need of required energy substrates; this is true even under normal glucose circulating levels. Therefore, under demanding situations energy substrates are provided by both glycolysis and glycogenolysis (Brown & Ransom, 2007). Conclusively, this supports the argument that changes in peripheral BG regulation are unlikely to reflect changes of glucose supply in the working brain areas.

2.2.2 Effects of Glucose on Cognitive Function and Mood

Studies examining the effects of breakfast omission on CF have provided proof in support of the assumption that the brain is vulnerable to the effects of short-term fasting (i.e. overnight fast), which seems to interfere with learning and cognition; to what extent, and under which mechanisms this remains to be elucidated. It has been hypothesized that the detrimental effects of omitting breakfast on cognitive performance may be mediated by the effects of CHO, and specifically glucose, on circulating glucose levels or by the supply of glucose to the brain. This is why the effects of glucose on CF, and the possible mechanisms mediating this effect, are going to be considered here.

There has been a great interest in the effects of glucose ingestion on CF, and of the resulting BG levels, rising or falling; relating either to omitting intake on the whole or ingesting a specific amount. This interest in CHO and their effects on CF has mainly arisen from the fact that the main source of energy for the CNS is glucose, and that hypoglycaemia can result in detrimental effects on performance and mood. The possible interactions between nutritional manipulations of CHO intake, physiological responses, psychological effects and CF are schematically presented in Figure 2.1 (Gibson, 2007). Each one of these factors and the potential mechanisms involved are discussed.

Figure 2.1: Pathways linking carbohydrate intake and mental function.



Source: Gibson (Gibson, 2007).

CHAPTER 2: LITERATURE REVIEW

An important confounder when assessing the effects of glucose on performance is the individual mental state (i.e. psychological state). The perceived difficulty, the emotional state the participants are under during the testing, the motivation and effort they put into doing the tests, how well they can cope under stressful conditions (i.e. CF testing), their vigilance and arousal, even how well and how long they have slept, are important factors that need to considered when interpreting results, and that could be responsible for the variability in findings (Westenhoefer *et al.*, 2004).

The effects of glucose on CF have been extensively reviewed elsewhere (Dye *et al.*, 2000; Gibson & Green, 2002; Messier, 2004; Riby, 2004; Hoyland *et al.*, 2008). Our focus is going to be healthy young adults, as this age group is closer to the one we are interested in (i.e. school children). In general, the findings so far suggest that there is a tendency for doses of 25g of glucose to be facilitating performance compared with a placebo drink, particularly affecting memory tasks (with the majority of the studies in elderly or diseased populations); improvements in performance have been associated with increased or falling BG levels; poor glucoregulators seem to be selectively facilitated by the administration of glucose; and task demand/ difficulty seems to be an important mediator of the glucose memory enhancing effects, especially in young adults. Each one of these findings is going to be briefly considered here, as well as the possible physiological and neurological processes that underlie these findings.

The effects of glucose on human memory appear to follow an inverted-U dose-response curve, where low doses have minimal or no effect, intermediate doses enhance memory, and higher doses either have no effect or impair memory; a dose of 25g appears to be optimal in healthy elderly people (Parsons & Gold, 1992). Glucose administration (usually 25-50g) when compared to a placebo drink (saccharin/ aspartame and/ or water treatment) seems to preferably influence memory tasks, while non-memory tasks (attention, motor speed, overall IQ) remain unaffected (Manning *et al.*, 1990). Specifically, in healthy young adults, where a non-prior memory deficit exists, declarative verbal memory (i.e. retention and retrieval from long-term memory) seems to be selectively enhanced by glucose administration (Foster *et al.*, 1998; Sunram-Lea *et al.*, 2001). Foster (Foster *et al.*, 1998) found that there was no glucose enhancing effect on immediate free recall (word list), digit count (forwards/ backwards – working memory performance, short-term verbal memory), long delay

recognition or the Rey-Osterrieth complex figure (long-term memory for non-verbal material). When the same design was repeated by the same researchers (Sunram-Lea *et al.*, 2001) this no-effect remained for the immediate free recall and the digit count. Similarly, Green (Green *et al.*, 2001) and Benton (Benton *et al.*, 1994) observed that the glucose treatment did not preferentially affect performance on an immediate recall task (total words remembered). Surprisingly, Scholey (Scholey *et al.*, 2001) found no glucose effect on a delayed word memory task; perhaps, this could be attributed to the different methodological design, where the testing began 45 min after glucose administration rather than the 20 min interval in previous studies (Foster *et al.*, 1998; Sunram-Lea *et al.*, 2001), and the glucose dose (25g *vs.* 50g, respectively).

Yet, declarative verbal memory as well other cognitive aspects have been shown to improve: reaction times in a recognition memory task but not in a two-finger tapping task (Green *et al.*, 2001), rapid information processing (Donohoe & Benton, 1999a; Green *et al.*, 2001), the Stroop paradigm (Benton *et al.*, 1994; Craft *et al.*, 1994), Porteus maze and verbal fluency (word generation) tasks (Donohoe & Benton, 1999a), the Rey-Osterrieth complex figure (Sunram-Lea *et al.*, 2001; Sunram-Lea *et al.*, 2002), serial subtractions (serial sevens) (Kennedy & Scholey, 2000; Scholey *et al.*, 2001; Sunram-Lea *et al.*, 2002). Donohoe and Benton (Donohoe & Benton, 1999a) found no selective glucose effect on a water jar test (problem solving), embedded figures test, a logical reasoning test, and a block design test. There was a tendency for improved performance on a verbal fluency task (word generation, declarative verbal memory) in the glucose condition, which did not reach statistical significance (Kennedy & Scholey, 2000; Scholey *et al.*, 2001); that could be attributed to not being as cognitively demanding as the one employed by Donohoe and Benton (Donohoe & Benton, 1999a). Benton (Benton *et al.*, 1994) did not find an effect on a rapid information processing task (attentional measure), contrary to the findings by Green (Green *et al.*, 2001), probably due to differences in methodological design. In the study by Benton subjects received 50g of glucose at baseline and then another 25g before the test (25 min later), while in the study by Green subjects only received 50g of glucose at baseline and performed the test 30 min later. Furthermore, Green (Green *et al.*, 2001) examined whether the expectancy concerning the nature of the substance (glucose *vs.* aspartame) can exert influences on task performance. Indeed, differences in performance in this attentional task (Bakan task) between the

glucose and the placebo group were only true when subjects were told they were given glucose.

Mnemonic processes seem to be preferentially influenced by the administration of glucose. When it comes to attention though, the results are conflicting. A study by Benton (Benton *et al.*, 1987) demonstrated that glucose improved attention and decreased reaction to frustration in normal children. The limited number of studies on attention in adults have provided conflicting results; studies have found no evidence of an impact (Manning *et al.*, 1990), a positive effect (Benton *et al.*, 1994), or even an impairment in attention (Flint, Jr. & Turek, 2003). The conflicting results are not surprising, because human attention is 'a highly complex phenomena comprised of multiple components, including but not limited to, stimulus detection, selective attention, divided attention, sustained attention (vigilance), working memory, and level of arousal' (Benton *et al.*, 1994).

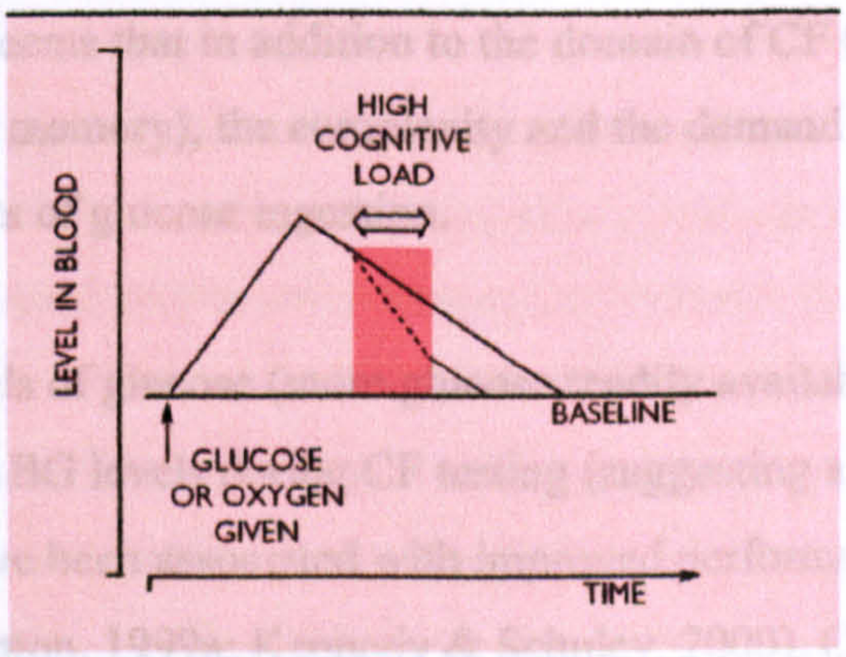
In general it could be said that mnemonic processes, like short- and long-term memory are improved by glucose administration, as well as performance on attentional and non-mnemonic tasks that require high mental effort and use of working memory for their completion (Gibson & Green, 2002; Hoyland *et al.*, 2008). Perhaps, as concluded by Kennedy and Scholey (Kennedy & Scholey, 2000) these tasks that are selectively affected may rely on working memory, which is mediated by the frontal lobes (i.e. higher executive functions).

The issue of higher **mental effort**[5], or as commonly known **cognitive/ task demand** on the effects of glucose on CF is going to be considered here, and is reflected by changes in brain metabolism, and falling BG levels. It appears that glucose administration can selectively benefit tasks that are cognitively demanding (Donohoe & Benton, 1999a; Benton & Nabb, 2003). Although it is difficult to quantify cognitive demand, the duration of the task and its complexity can be considered. As explained by Scholey (Scholey, 2001), there are two ways to assess cognitive demand, which

[5] 'Mental effort or cognitive demand involves aspects of memory load, intensity of procedures and other features that make a task more difficult to perform' (Scholey, 2001).

allow for a comparison among the tests administered: physiological arousal, which increases in intense cognitive processing (i.e. faster breathing, increased heart rate, widening of blood vessels, release of glucose); and questionnaires addressing the issue of task demand and effort (which is what was used in the present PhD project). During a cognitive demanding task both glucose and oxygen in the brain are 'used up' faster; the same has been argued about glucose and oxygen levels in blood (Figure 2.2).

Figure 2.2: The impact of a cognitive load on glucose and oxygen levels in the blood.



Source: Scholey (Scholey, 2001).

It seems that the more demanding a task is, the more susceptible to a beneficial effect of glucose administration it is. The provision of glucose has been found to facilitate the difficult rather than the easier trials of the Stroop test (Benton *et al.*, 1994), while falling BG levels were associated with lower self-reported energy when participants had to perform three demanding tasks in succession (Owens *et al.*, 1997). A glucose drink selectively benefited the more difficult tests of the Porteus Maze (Donohoe & Benton, 1999a). When the duration of the task was considered, glucose benefited a vigilance task, but only towards the end of the test session (Benton & Owens, 1993) Thus, it could be argued that the later stages of prolonged tasks are susceptible to the provision of glucose.

Scholey (Scholey *et al.*, 2001) demonstrated that the level of demand rather than the type of cognitive domain primarily determines its susceptibility to a glucose enhancement effect. Glucose consumption significantly improved performance on serial sevens, with a trend for improved performance on word retrieval, and no effect

on the word memory task. Since serial sevens is considered as the most cognitive demanding task of the three, the degree of the cognitive demand seems to be an important factor determining the extent to which task performance may be enhanced by glucose (Kennedy & Scholey, 2000; Scholey *et al.*, 2001). Similarly, two more recent studies showed that glucose administration improved performance in verbal memory tasks, but only in the more difficult ones (Sunram-Lea *et al.*, 2002; Meikle *et al.*, 2005). Nonetheless, the influence of task demand seems to be subjective as it can be influenced by perceived difficulty and effort, arousal and engagement, and could also be affected by age, personal traits, and glucoregulation (Gibson & Green, 2002; Riby *et al.*, 2006). It seems that in addition to the domain of CF (particularly hippocampal episodic memory), the complexity and the demand of a cognitive test is crucial to detect effects of glucose ingestion.

Elevated pre-task levels of glucose (more glucose readily available before the CF testing), and a drop in BG levels during CF testing (suggesting a greater utilization or uptake of glucose) have been associated with improved performance (Benton *et al.*, 1994; Donohoe & Benton, 1999a; Kennedy & Scholey, 2000). Cognitively demanding tasks appear to be associated with a greater fall in BG levels compared with less demanding tasks. Indeed, Scholey (Scholey *et al.*, 2001) reported a greater fall in BG levels after the ingestion of 25g of glucose during a serial sevens subtraction task, rather than during the less demanding key-tapping control task. Similarly, BG levels were significantly lower during the incongruent compared to the congruent condition of a Stroop task, while BG levels fell throughout the testing for both conditions (Fairclough & Houston, 2004).

In fact, given the clear evidence from PET (Positron Emission Tomography) and functional MRI (Magnetic Reasoning Imaging) studies that increased cognitive demand is associated with an increased use of glucose by the brain (Hoge & Pike, 2001), it is perhaps not surprising that an increased supply of glucose benefits more demanding tasks. The question is whether individual differences in the ability to adequately supply glucose to very active parts of the brain will influence memory under cognitively demanding situations (Donohoe & Benton, 2000). The brain has been thought to be adequately supplied with glucose, resulting from a uniform concentration of extracellular glucose. However, this view has been challenged, and

studies in animals have revealed that the concentration of extra-cellular glucose varies with the strain of the rat and the area of the brain examined (McNay & Gold, 1999).

In freely moving rats McNay and Gold (McNay & Gold, 1997) showed a 25% decrease in hippocampal extracellular glucose during a spatial working memory task, suggesting that the hippocampus is unique in terms of requiring greater amounts of glucose. A more recent study by the same researchers revealed that the decrease in extracellular glucose was higher during the more demanding spatial task (McNay *et al.*, 2000). An injection of glucose, but not placebo, prevented this decline in glucose in hippocampus, which is thought to be involved in declarative memory tasks. Nonetheless, these changes in extracellular glucose were not attributable to changes in BG levels, as peripheral BG increased (McNay *et al.*, 2001); prior glucose administration decreased this increase. Perhaps, this increase, contrary to what has been observed in human adults, could be attributed to adrenergic arousal due to higher energy demands (Gibson, 2007). In support of this hypothesis are results in human adults, where an increase in BG levels has been observed after glucose administration during more demanding tasks (Sunram-Lea *et al.*, 2002). It seems that the brain's metabolism of glucose, at least in selective areas, increases during cognitively demanding tasks. Nonetheless, changes in peripheral BG levels are unlikely to reflect changes of glucose supply in the working brain areas (Messier, 2004). Besides, astrocytic glycogen is readily available under cognitive functioning to cover local demand (Brown & Ransom, 2007).

Interpretation of the findings becomes quite difficult, due to the several experimental designs employed, i.e. different dose of glucose, different timing, different tasks employed, different pre-nutritional status (short-term: hours of fasting, meal the night before, etc; long-term: nutritional status). Nonetheless, another important factor that seems to be mediating the glucose enhancing effect is glucoregulation. Peripheral glucose regulation can influence memory performance not only in elderly people (Messier *et al.*, 1997), but as recent data imply in healthy young adults as well (Messier *et al.*, 1999; Donohoe & Benton, 2000; Awad *et al.*, 2002). Students with poorer glucoregulation (ability to deal with a glucose load; higher BG rise after a glucose load) performed worse in tests of word free recall, both immediate and delayed; this effect was alleviated by the ingestion of 50g of glucose (Messier *et al.*,

1999). Similarly, Awad (Awad *et al.*, 2002) showed that performance was worse in poor glucoregulators when verbal declarative tasks were administered, effect though which was alleviated by the ingestion of 75g of glucose only for the most difficult task. Moreover, Donohoe and Benton (Donohoe & Benton, 2000) observed that between two to three hours after the ingestion of 50g of glucose, the quicker BG returned to baseline after it had reached the lowest value, the better the performance was for both immediate and delayed recall. The question arises as to whether in healthy young adults with poor glucoregulation improvements in glucose tolerance, as for example through foods or diets would be associated with improved cognitive functioning.

It has also been observed both in young (Awad *et al.*, 2002) and elderly (Messier *et al.*, 2003) subjects that the glucose load (75g and 50g, respectively) restored the memory deficit in poor glucoregulators when given the saccharin treatment; this effect was not observed in good glucoregulators. Similarly, ingestion of 50g of available CHO (glucose, potato, or barley) improved performance on verbal declarative tasks (immediate and delayed paragraph recall) and a visuomotor task in elderly subjects with poor pancreatic β-cell function (Kaplan *et al.*, 2000); effect which was independent of plasma glucose levels. As suggested by Gibson (Gibson, 2007), it could be that in poor glucoregulators there is observed a shift of the inverted-U dose-response curve to the right, where higher doses of glucose (50 or 75g), 'which might be ineffective or even impairing in good glucoregulators, now become more optimal in poor glucoregulators'.

Overall, it seems that the glucose enhancing effect in mnemonic processes (especially declarative verbal tasks) is more consistent than the glucose effects on non-mnemonic tasks. Nonetheless, as concluded in a recent review, a majority of tasks within a certain domain have to be employed before it can be suggested that a specific macronutrient has no effect on performance (Hoyland *et al.*, 2008). Any benefits of glucose administration in young adults are most likely to be observed only when participants are engaged in sufficiently demanding cognitive tasks. This beneficial effect has been demonstrated as a direct consequence of a glucose load or in relation to the extent of change in BG following the load.

2.2.3 Physiological and neurological mechanisms mediating the effect of Glucose on Cognitive Function

Four potential mechanisms have been proposed to mediate the effects of glucose on CF: adrenocortical activity, sympathetic arousal, neuronal metabolism and energy supply, and neurotransmitter synthesis. These mechanisms have been extensively reviewed by Gibson (Gibson & Green, 2002; Gibson, 2007). Therefore, they will be mentioned only briefly, with more emphasis on the first one (as it is central to our hypothesis), referring to the key issues surrounding their effects.

❖ **Adrenocortical activity and glucoregulation**

It was mentioned in the previous chapter that glucoregulation is involved in the memory enhancing effects of glucose not only in the elderly (Kaplan *et al.*, 2000), but as recent research suggests in healthy young adults as well, after a challenging task (Donohoe & Benton, 1999a). Gibson and Green (Gibson & Green, 2002) suggested that the glucocorticoid hormone, cortisol, may be mediating this effect. Cortisol is secreted in response to stress through the HPA-axis, and is also an important counter-regulatory hormone involved in the regulation of peripheral glucose mobilization and metabolism. Similar to glucose, there seems to be an inverted-U relationship between cortisol dose and cognitive performance, especially memory (Abercrombie *et al.*, 2003). Cortisol seems to be involved in the memory recall enhancement of 'emotional stimuli' (amygdala is involved) (Buchanan & Lovallo, 2001), and the impairment of 'neutral stimuli' (hippocampus is involved), such as declarative verbal memory, and working memory (Kirschbaum *et al.*, 1996; Lupien *et al.*, 1999).

In the presence of a stressful task, a rise in cortisol levels would be expected. Administration or absence of a glucose load prior to the stressful task could have an effect on the cortisol rise; fasting did not induce a rise in cortisol and stress had to be present for the glucose mediated release of cortisol to take place (Kirschbaum *et al.*, 1997). Therefore, it could be argued that low BG levels can perhaps prevent the activation of the HPA-axis and subsequent cortisol release. Recently, it has been shown that this effect is specific to glucose, when compared to a fat or protein load (Gonzalez-Bono *et al.*, 2002). These findings might explain why in some cases children who missed breakfast performed better on memory tasks (Benton & Owens,

1993), and why poor glucoregulators exhibit poor memory performance under demanding CF testing (Messier, 2004). Indeed, recent evidence suggests that subjects with glucose intolerance have significantly higher morning cortisol levels (Reynolds *et al.*, 2001), and respond greater to stress (higher cortisol release) (Rosmond & Bjorntorp, 2000).

One possible explanation is that faster uptake and disposal of glucose in good glucoregulators would result in a smaller rise in cortisol levels during a challenging task, and as such would minimize the detrimental effects of cortisol on performance. This explains earlier findings where falling glucose levels and increased insulin secretion predicted performance (Gibson & Green, 2002); and the recent findings that in poor glucoregulators poor performance is ameliorated after a glucose load (Awad *et al.*, 2002). Cortisol has been found to inhibit glucose transport in both hippocampal neurons and astrocytes, in line with the cortisol inhibition of glucose transport in peripheral tissues (Virgin, Jr. *et al.*, 1991). It could be that higher glucose levels, and as a result higher insulin levels, reverse this inhibition. Further work is needed to establish the role of insulin in cognition, since the identification of insulin and insulin receptors in the brain suggests that the brain is a target organ for insulin (Zhao *et al.*, 2004). These insulin receptors are expressed in the limbic system, and especially in the hippocampus and the cerebral cortex, an area important for learning and memory. The potential role of insulin in the observed effects of glucose 'remains largely unexplored, due to the inherent difficulty of separating the action of insulin from that of glucose' (Messier, 2004). Nonetheless, systemic infusion of insulin while maintaining euglycaemic levels (i.e. to avoid the memory impairment associated with hypoglycaemia) was associated with improved performance in verbal memory and selective attention (Kern *et al.*, 2001). As such, it could be argued that in poor glucoregulators when given a glucose load (i.e. maintaining euglycaemic conditions), the higher insulin levels could benefit memory performance. As concluded by Gibson and Green (Gibson & Green, 2002), 'the critical interaction is likely to be between glucoregulatory processes and the impact of a challenging task'.

❖ Sympathetic arousal

Gibson (Gibson, 2007) suggested that ingestion of glucose (especially under demanding tasks) may elicit sympathetic arousal, and as such result in better performance; poor glucoregulators may particularly benefit from this effect. A number of experiments support the idea that adrenergic arousal and hormonal changes associated with it, can enhance memory and raise BG as well (Gibson & Green, 2002). Nonetheless, the latter is mainly mediated by inhibiting the uptake and disposal of glucose under acute psychological stress (Wiesli *et al.*, 2005), although arousal by a challenging task does not necessarily result in an increase in BG levels (Green *et al.*, 1997). Furthermore, glucose, or other food has been shown to educe sympathetic activation (Macht, 1996; Kennedy & Scholey, 2000).

The effect of arousal during a demanding task and after a glucose load on subsequent glucose levels and resulting performance is quite complex, as there are many issues involved. Not all subjects necessarily react the same way to stressful stimuli, depending on how demanding they perceive the challenging task to be (Messier, 2004); that over-arousal might indeed be detrimental to performance (Reid M & Hammersley R, 1999); that rising BG levels could be a sign of less efficient cell uptake and disposal from the circulation rather than arousal; that pre-task BG levels could correlate positively with performance, whether or not glucose was ingested; and that heart rate could also correlate with performance levels (Kennedy & Scholey, 2000).

❖ Neuronal metabolism

As explained in section 2.2.1 (page 40) astrocytes respond to neuronal activity (i.e. increased oxygen and glucose supply to the working area, through increased blood flow), and through their stored glycogen protect the brain against reductions in its main energy substrate, glucose. This explains why there are only subtle effects of glucose/ CHO administration on CF. Still, there have been observed effects, suggesting that there might be an association between circulating glucose levels and neuronal glucose metabolism. For example, in mice a reduction in hippocampal extracellular glucose during neuronal activity was prevented by glucose administration, suggesting that this area of the brain might be vulnerable (McNay *et al.*, 2001). Nonetheless, the mechanisms or the specific glucose dose that can elicit the

effect remain to be elucidated. A new theory has been proposed, 'The Selfish Brain Theory', which suggests that the brain gives priority to its own energy supply, by controlling both local and peripheral metabolism to meet its energy needs (Peters *et al.*, 2004). Gibson (Gibson, 2007) suggested that two aspects of this theory might be involved in the observed glucose enhancing CF effects. Firstly, when local demand increases, as expressed by low energy levels (i.e. ATP – adenosine triphosphate), excitatory neurons give the signal for more energy, but when this energy need can be covered by a glucose load for example, then inhibitory neurons prevail. This might explain the narrow dose seen for the glucose enhancing effect. Secondly, this theory suggests that the HPA-axis might also be involved through cortisol secretion; HPA activation at first increases the supply of energy to the brain, which is nonetheless inhibited when under high cortisol levels. Based on these two aspects of this theory, it could be argued that administration of a glucose load under stressful conditions (e.g. CF testing) could result in such a cortisol rise which could restrict the energy supply to the brain, and that the hippocampus might be sensitive to this effect (Gibson, 2007).

❖ **Neurotransmitter synthesis**

Glucose has been implicated in the activity of three neurotransmitters: glutamate, acetylcholine, and serotonin (reviewed by Gibson (Gibson, 2007)). The association with glutamate has been inferred from the observation that drugs which release glutamate (such as nicotine) have similar effects to glucose, that is memory enhancing effects during demanding tasks, and subtle improvements in mood. Glucose availability has been also associated with increased release of acetylcholine, especially in the hippocampus. Acetylcholine is formed by choline and acetyl-CoA. The enzyme mediating the synthesis of acetylcholine (choline acetyltranferase) is not saturated, suggesting that it depends on a continuous supply of acetyl-CoA, which is in part a by-product of glucose metabolism (Messier, 2004). Nonetheless, while glucose availability can influence the production of acetylcholine, more complicated mechanisms would need to be invoked for an effect of plasma glucose on the synthesis and release of acetylcholine to take place (Gibson & Green, 2002). Studies in animals, suggest that although glucose does not facilitate an increase in acetylcholine under non-stressful conditions, when there is increased demand for its synthesis (i.e. demanding tasks), increased glucose availability results in a greater

synthesis of acetylcholine and subsequent improved performance (Messier *et al.*, 1990). Nonetheless, as concluded by Messier (Messier, 2004) it is difficult to elucidate the mechanisms involved, as there seems to be a narrow dose glucose effect, where certain glucose doses that increase BG levels do not improve memory or increase acetylcholine synthesis.

Finally, carbohydrates (glucose) have been related to mood, mainly through their ability to affect serotonin levels, by increasing the availability of TRP to the brain. There is a well established link between serotonin, cognition, mood and behaviour, including depression, aggression, and impulsivity, even lethargy/ sleep (Gibson & Green, 2002). The synthesis of serotonin (5-hydroxytryptamin, 5-HT) depends on the dietary supply of the precursor essential amino acid TRP, due to a lack of saturation of the rate-limiting enzyme, tryptophan hydroxylase (Fernstrom, 1983). Tryptophan competes with the large neutral amino acids (LNAA: tyrosine, phenylalanine, leucine, isoleucine) for the transport to the brain. A meal high in CHO results in an increase in BG levels, followed by the release of insulin. Insulin, causes the LNAAs to be taken up into muscle, resulting in an increase in the ratio of TRP to LNAA, and thus more TRP is transported into the brain; there, it is metabolized in the neurotransmitter, serotonin. Nonetheless, even as little as 5% of total energy as protein can prevent this phenomenon (Fernstrom & Fernstrom, 1995). Besides, in humans, even when the level of TRP increases, this is not related to an increased release of serotonin (Benton, 2002). Thus, when a normal diet is consumed, the implication of this underlying mechanism becomes questionable. It seems that stress, and as a result of that cortisol (HPA-axis) might be involved in this mechanism. Interestingly enough, the induced rise in TRP by a high CHO very low protein meal protected stress-prone individuals from a deterioration in mood (Markus *et al.*, 1998). It could well be that hyperglycaemia and hyperinsulinaemia observed not only in stress-prone or depressed individuals, but also in poor glucoregulators, increases susceptibility to dietary manipulations of serotonin (Gibson, 2007).

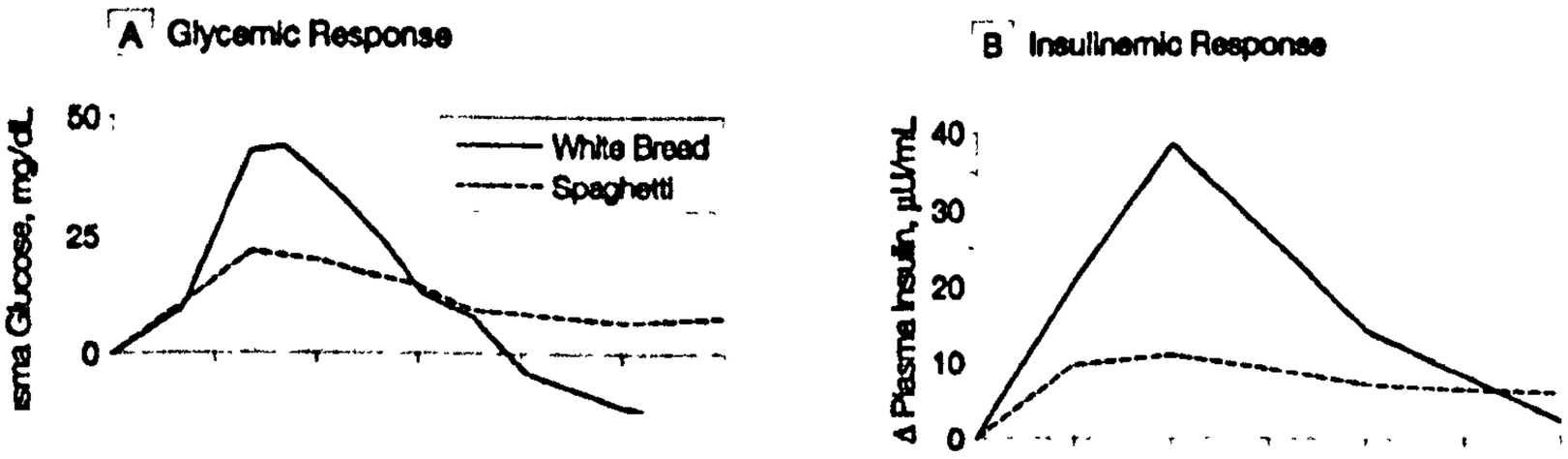
2.3 Glycaemic Index, Glycaemic Load, Cognitive Function and Mood

2.3.1 Definition of Glycaemic Index and Glycaemic Load

Studies looking into the glucose effects on CF could form the basis for the hypothesis that glucose may be mediating the memory enhancing effects of breakfast. Nonetheless, pure sources of glucose will be rarely consumed as part of a balanced diet. Therefore, the possibility that CHO-containing foods/ meals with different glycaemic responses could exert different effects on CF should be considered. The effect of CHO-containing foods on BG can be assessed by the use of GI and GL.

The concept of the GI was first introduced by Jenkins (Jenkins *et al.*, 1981) as a system for classifying equal amounts of CHO-foods according to their glycaemic response, usually having an energy content of more than 80% from CHO; and as a tool for exchanging one source of CHO for another, so that the overall macronutrient composition remains about the same. It is a measure of digestion and absorption of CHO-foods and the resultant effect on the BG and insulin levels, and it does not change with food or CHO intake. When foods with equal amounts of available CHO are compared, foods with a high GI produce a higher peak in postprandial BG and a greater overall BG response during the first two hours after consumption compared with foods with a low GI (Figure 2.3). Furthermore, they induce a greater rise and fall in blood insulin, leading to lower concentrations of the body's two main fuels (BG and fatty acids) in the immediate postabsorptive period (Ludwig, 2002).

Figure 2.3: Glycaemic and Insulinaemic responses after ingestion of carbohydrates with different Glycaemic Indexes.



Source: Ludwig (Ludwig, 2002) (White Bread: High GI food; Spaghetti: Low GI food).

According to the FAO/ WHO, the GI of a certain food is defined as 'the incremental area under the blood glucose response curve (iAUC) of a 50g CHO portion of a test food expressed as percent of the response to the same amount of CHO from a standard food taken by the same subject'. The iAUC includes only the area above the fasting level, and capillary blood is used to measure it by applying the trapezoid rule (FAO/WHO, 1998). Therefore, by definition the GI is an *in vivo* measurement of the glycaemic response, and *in vitro* measurements should not be used to indicate the GI of foods (Brand-Miller & Holt, 2004). The iAUC is the recommended method for measuring glycaemic, and subsequently insulinaemic responses (FAO/WHO, 1998). Indeed, this method yields more valid and/ or more precise GI values than seven other methods, as recently tested by Wolever (Wolever, 2004). The capillary blood is preferred in comparison to the venous blood, because it is easier to obtain, the rise in BG is greater, and the results are less variable (i.e. reduces within-subject variation); thus, statistically greater differences are easier to be detected (FAO/WHO, 1998; Wolever *et al.*, 2003; Wolever, 2004). In fact, the FAO/ WHO recommends that the concept of the glycaemic CHO, meaning 'providing energy for metabolism' should be adopted, and used in conjunction with information about food composition to guide food choices. Furthermore, the 'bulk of the CHO-containing foods consumed should be rich in non-starch polysaccharides and with a low GI'. It is apparent that a standardized way of measuring glycaemic responses and calculating GI values is required, which has recently been reviewed and proposed to ensure quality of results; the general formula for calculating iAUC is also presented (Brouns *et al.*, 2005). It is recommended that the GI values of foods should be expressed relative to glucose, for international standardization (inter-laboratory standard deviation (SD) of GI values is approximately nine) (Wolever *et al.*, 2003).

Glycaemic index by definition can be used to compare equal amounts of CHO, and as such is a measure of CHO quality (type of CHO) and not quantity (amount). Therefore, the GI is not a reliable tool to guide food choices when used on its own. 'Both the quality and the type or source of CHO found in foods influence postprandial glucose level' (ADA, 2004). Indeed, the amount and GI of CHO account for ~90% of the total variability in mean BG and insulin responses (Wolever & Bolognesi, 1996a; Wolever & Bolognesi, 1996b; Wolever *et al.*, 2006).

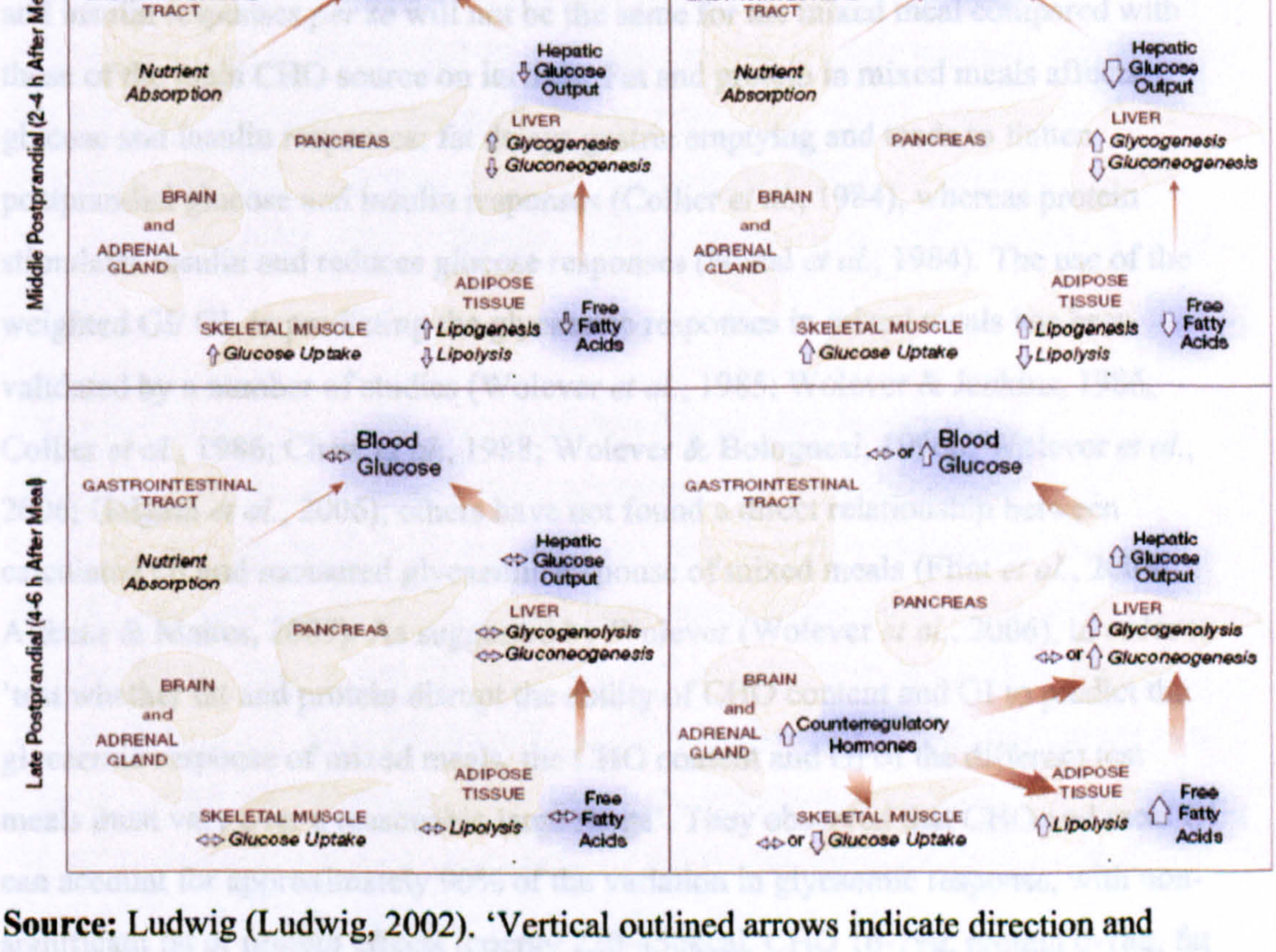
The glycaemic load (GL) is a concept that takes into account both the quantity and the type of CHO (Salmeron *et al.*, 1997). It is the product of a food's GI and its total available CHO content: GL=[GI x CHO (g)]/ 100; it has been validated and it predicts BG and/ or response in an approximately linear fashion – in the normal range of available CHO intakes (15-100g) (Brand-Miller *et al.*, 2003); and it is numerically the same as the GGE (glycaemic glucose equivalent), which is the weight of glucose (g) that would induce a glycaemic response equal to that induced by the food (Monro & Williams, 2000; Liu *et al.*, 2003). Specifically, Liu (Liu *et al.*, 2003) reported that foods administered at the same GGE dose produce similar glycaemic responses despite up to a 2.5-fold range in CHO content, and that doubling the dose doubles the response.

From the mathematical equation used to calculate GL, it can be seen that a low GI – high CHO food/ meal and a high GI – low CHO food/ meal will produce the same GL. However, while their effects on postprandial glycaemic response (i.e. iAUC) may be similar, the metabolic effects produced by the two foods will be very different (Ludwig, 2002; Barclay *et al.*, 2005). The differences in the metabolic responses between a high and a low GI meal are presented in Figure 2.4 (Ludwig, 2002). In summary, in the early postprandial period (0-2 hours after the meal) the high GI meal results in higher BG levels and an increased insulin-to-glucagon ratio, compared with a low GI meal of similar energy and nutrient content; thus, resulting in stimulation of glycogenesis and lipogenesis and suppression of gluconeogenesis and lipolysis. In the middle postprandial period (2-4 hours after the meal) the high GI meal is associated with increased insulin and decreased glucagon levels, which elicit a decrease in BG levels, often below preprandial levels; free fatty acids' concentration remains suppressed. Finally, in the late postprandial period (4-6 hours after the meal) counter-regulatory hormones result in an increase in the two metabolic fuels, resembling a state of many hours of fasting. On the contrary, hypoglycaemia (<2.2mmol/L) does not take place after the low GI meal.

Concluding, GI and GL should never be used in isolation, but in conjunction when determining the glycaemic potency of a food or a meal. There is a proposed GI and GL range (not universally accepted) where GI values ≤55 are low, between 56-69 are

medium, and ≥70 are high; and GL values ≤10 are low, between 11-19 are medium, and ≥20 are high (Brand-Miller *et al.*, 2003).

Figure 2.4: Metabolic responses following ingestion of a high GI meal compared with a low GI meal.



Source: Ludwig (Ludwig, 2002). 'Vertical outlined arrows indicate direction and magnitude of change from baseline (preprandial) state indicated by horizontal outlined arrows'.

2.3.2 Application of Glycaemic Index and Glycaemic Load to mixed meals

The GI measures the glycaemic response after the consumption of a certain CHO food. But what happens in mixed meals? In mixed meals, contrary to individual foods, the GI is not measured, it is calculated; what is measured is the glycaemic response, as represented by the iAUC two hours after the ingestion of the meal. In mixed meals or diets, the GI is calculated as the weighted average of the GI of each food with the weighting based on the proportion of total meal CHO contributed by the foods (g of CHO in food to total g of CHO in meal) (Wolever & Jenkins, 1986). This effectively means that if for example there are three foods in the meal (A, B, C), then the meal GI (MGI) is $MGI=MGI_A+MGI_B+MGI_C$ ($MGI_{A/B/C}=g_{A/B/C}/g_{total}$). The GL is calculated by the sum of all the GL of all foods in the meal or diet $GL=\Sigma_{foods}$[GI x (CHO/portion) x no of portions per day/100] (Salmeron *et al.*, 1997)

The most important issue when applying GI to mixed meals is the co-ingestion of other macronutrients. When the main CHO source (food) is part of a meal, the glucose and insulin responses *per se* will not be the same for the mixed meal compared with those of the main CHO source on its own. Fat and protein in mixed meals affects glucose and insulin responses: fat delays gastric emptying and tends to flatten postprandial glucose and insulin responses (Collier *et al.*, 1984), whereas protein stimulates insulin and reduces glucose responses (Nuttal *et al.*, 1984). The use of the weighted GI/ GL in predicting the glycaemic responses in mixed meals has been validated by a number of studies (Wolever *et al.*, 1985; Wolever & Jenkins, 1986; Collier *et al.*, 1986; Chew *et al.*, 1988; Wolever & Bolognesi, 1996b; Wolever *et al.*, 2006; Galgani *et al.*, 2006); others have not found a direct relationship between calculated GI and measured glycaemic response of mixed meals (Flint *et al.*, 2004; Alfenas & Mattes, 2005). As suggested by Wolever (Wolever *et al.*, 2006), in order to 'test whether fat and protein disrupt the ability of CHO content and GI to predict the glycaemic response of mixed meals, the CHO content and GI of the different test meals must vary over a reasonable large range'. They observed that CHO and meal GI can account for approximately 90% of the variation in glycaemic response, with non-significant fat or protein effects (energy 220-450kcal, CHO 16-79g, protein 0-18g, fat 0-18 g). According to the same authors misclassification of the GI values, the fixed CHO content to 50g and the large coefficient of variation (CV) for protein (51%) and

fat (89%) is why Flint (Flint *et al.*, 2004) did not observe an effect; furthermore, the small number of subjects used to test each food (n=3), and the large 95% confidence interval (CI) of the measured GI values (±70) can account for the non-observed effects by Alfenas and Mattes (Alfenas & Mattes, 2005). It seems that more studies are in support of the application of the GI concept, and subsequently GL in mixed meals and diets. Specifically, the FAO/ WHO (FAO/WHO, 1998) accepts this prediction model, and states that 'the GI can be applied in a detailed fashion to mixed meals or whole diets by calculating the GI value of the meal or diet'.

2.3.3 Effects of Glycaemic Index and Glycaemic Load on Cognitive Function and Mood

The GI and GL have been extensively used as tools in studies assessing disease risk associated with dietary CHO intake, such as diabetes (Pi-Sunyer, 2005), cardiovascular diseases (Ebbeling *et al.*, 2005) and obesity (Millan-Price & Brand-Miller, 2006). The use of the GI as a tool to assess the effects of the glycaemic potency of a food or mixed meal on cognition has only recently emerged, and as such has not been thoroughly investigated. To my knowledge, only five studies so far have considered this issue in young adults and school-aged children (Benton *et al.*, 2003; Mahoney *et al.*, 2005; Nabb & Benton, 2006; Ingwersen *et al.*, 2007; Benton & Jarvis, 2007).

Wesnes (Wesnes *et al.*, 2003) compared the effects of two breakfast cereals (shreddies, cheerios, served with milk) with those of a glucose drink and a no breakfast condition in 21 children aged 9-16 years; shreddies and glucose had 38.3g of CHO, and cheerios 28.7g. Glycaemic index was not considered in this study between the two cereals, but glucose could well be considered as a high GI treatment. This study supports the beneficial effect of CHO in the form of breakfast cereals over a glucose drink and the non-breakfast condition. Attention, working memory, episodic secondary memory, and mood were assessed prior to breakfast and then again 30, 90, 150, and 210 min later. Attention and episodic memory declined progressively throughout the morning, but to a much greater extent after the glucose than after the two cereals; performance after the glucose treatment was initially worse than after the no-breakfast. Mood was better and satiety was preserved after the cereals.

In 71 healthy young females (mean age 21y±1m), a low GI breakfast cereal (GI: 42) improved performance in the difficult abstract words of a word recall task 150 to 210 min after breakfast, but not earlier (30 and 90 min); this was when BG had returned to baseline, and was not different between the two cereals (Benton *et al.*, 2003). On the contrary, with the high GI cereal (greater plasma glucose rise, GI: 66) performance declined progressively throughout the morning. Energy and macronutrient content was similar for the two breakfast cereals. In a subsequent study by the same group in 189 young females (20-25 years old), memory (immediate and delayed), vigilance (rapid information processing task) and reaction times were assessed 30, 75 and 120 min after breakfasts varying in macronutrient content in young adults. The meals that produced the lowest average BG (low in protein, and low in both CHO and fat) were associated with better memory in better glucoregulators (as assessed by baseline BG levels). On the contrary, reaction times and vigilance were better after the high GL *vs.* the low GL meals, but only in people with better glucose tolerance (Nabb & Benton, 2006).

The study in school-aged children (Mahoney *et al.*, 2005) included two experiments, one in 9-11 year olds (15 males, 15 females), and the second in 6-8 year olds (15 males, 15 females). The two experiments had the same cross-over design, where subjects consumed either instant oatmeal (low GI), ready-to-eat cereal (high GI), or no breakfast. Spatial memory and short-term memory (in girls only) in both age groups and auditory attention in 6-8 year olds were improved one hour after the low GI breakfast compared with the high GI or the no breakfast. Moreover, there was no meal effect on overall mood as assessed at baseline, before and after the testing.

Ingwersen (Ingwersen *et al.*, 2007) found that in 6-11 year olds (n=64) the decline in accuracy of attention and secondary memory two hours after breakfast administration was improved in the low GI (All Bran, GI=42) *vs.* the high GI cereal (Coco Pops, GI=77). In either measure there was no effect of gender. The study did not control for GL, and in addition the macronutrient composition of the meals was different (High GI cereal: 133 kcal, Low GI cereal: 98 kcal; 36% difference in energy alone). To test if GI alone is having an effect, the nutritional (fat, protein, carbohydrate) composition of the meals should be kept the same (or similar), and only the source of the carbohydrate should be varied.

In a recent study by Benton (Benton *et al.*, 2007) three breakfast meals differing in their GL, but with similar macronutrient composition were administered in 19 children (10 girls, 9 boys) aged 6-7 years. Behaviour and CF were assessed two and a half hours after breakfast. There were no meal main effects with regard to memory (recall of objects, spatial memory) or the ability to sustain attention. Nonetheless, the GL of breakfast negatively correlated with performance; the lower the GL the better the memory performance and the ability to sustain attention.

These studies have provided some basis for the understanding of the underlying mechanisms. Gibson (Gibson, 2007) suggested that the observed differences in performance between high and low GI meals could be attributed to the HPA-axis; that is, the high GI (glucose is a high GI treatment) meals during challenging tasks, by increasing glucose levels and cortisol secretion, might result in an impairment of performance. Indeed, the association between the high GI (higher BG levels) and poor memory performance in the study by Benton (Benton *et al.*, 2003) and Ingwersen (Ingwersen *et al.*, 2007), could be attributed to increased cortisol secretion. Similarly, higher BG levels were associated with poorer memory performance (Nabb & Benton, 2006). These observations seem to be consistent where memory is concerned. On the contrary, reaction times were found to be worse after the low GI meal, though in good glucoregulators only (Nabb & Benton, 2006). Gibson (Gibson, 2007) reported the findings from an unpublished study of his group, which are similar to the former. Specifically, they found that reaction times on a Stroop colour-word task were faster after an overnight fast, followed by the high GI (cornflakes) and then the low GI treatment (muesli). These positive effects of fasting on this CF domain (speed of performance) are similar to those previously reported by Benton and Owens (Benton & Owens, 1993) and Green (Green *et al.*, 1997). Gibson (Gibson, 2007) suggested that different levels of arousal may be responsible for the different effects on CF; they found (unpublished results) that the fasting condition was associated with feeling the least relaxed after the CF tests, followed by those receiving the high GI treatment, while those in the low GI were as relaxed as before. These observations and the assumptions for a cortisol effect are supported by two studies were cortisol improved reaction times (Lupien *et al.*, 2002) and reduced errors of commission in word recall tests (Abercrombie *et al.*, 2003). Similarly, Gibson (Gibson, 2007) observed fewer errors of commission after the cornflakes than after the muesli (unpublished findings).

Therefore, it was hypothesized by Gibson (Gibson, 2007) that the higher GL in the cornflakes treatment could be associated with increased arousal (e.g. feeling less relaxed) under demanding tasks, higher cortisol levels, and subsequently better performance on this cognitive process. Still, as the fasting was associated with fewer errors of commission compared with the muesli treatment, it was proposed that other mechanisms could well be in place (Gibson, 2007).

The studies reported in this section are the first ones of their kind to address a possible role for breakfast meals differing in their GI and GL indices on CF in young adults and school children. Though the results are promising, clearly more well-controlled studies are needed in order for the role of GI and GL on cognition to be clarified, and for the possible mechanisms to be examined, especially in the adolescent age group, where no studies have been reported so far. Nonetheless, it seems to be emerging from these recent findings that the interaction between glucoregulatory processes and differences in cortisol secretion under stressful tasks could be the underlying mechanism. This helps to explain the observed results across the studies reported in this section.

2.3.4 Limitations

The same limitations apply to both GI and GL, as GL is a mathematical concept based on the GI approach of classifying CHO-containing foods. There are various food factors/ properties that could influence the GI of a certain food. These have been summarized elsewhere (Arvidsson-Lenner *et al.*, 2004); such as the type of starch (amylose *vs.* amylopectin), the physical form in which the food is eaten (juice *vs.* whole fruit, mashed potato *vs.* whole potato), the preparation of the food (cooking method, time), ripeness, degree of processing, type or variety of the food.
Furthermore, apart from these intrinsic factors, there are also extrinsic factors that could affect GI, such as the protein and fat eaten with the CHO food (see section 2.3.2, page 59), prior food intake (second-meal effect), as a low GI meal the evening before produces better glucose tolerance the following morning compared with a high GI evening meal (Wolever *et al.*, 1988; Granfeldt *et al.*, 2006), fasting or preprandial glucose levels, and degree of insulin resistance (Wolever & Bolognesi, 1996a; Foster-Powell *et al.*, 2002; Pi-Sunyer, 2002). The variation in published GI values for

apparently similar foods may reflect both methodological factors and true differences in the physical and chemical characteristics of the food. Indeed, GI values for processed foods may change over time if manufacturers make changes in the ingredients or processing methods used (Flint *et al.*, 2004).

Another important issue when considering the use of GI is that in the recommended GI determination, the available CHO is measured as total CHO minus dietary fibre analysed by the Association of Official Analytical Chemists (AOAC) method (FAO/WHO, 1998). Nonetheless, according to the current determination of dietary fibre, all indigestible CHO are considered dietary fibre, including resistant starch, non-digestible oligosaccharides and sugar alcohols (polyols) (Champ *et al.*, 2003). As the classical method did not measure these CHO appropriately, for products rich for example in resistant starch, the by-difference approach may overestimate the available CHO. Therefore, the question arises as to whether the currently published GI values of foods have been influenced by this (Foster-Powell *et al.*, 2002). Still, Brouns (Brouns *et al.*, 2005) concluded that the majority of foods in the International Table of GI and GL values does not include high levels of the unaccounted indigestible CHO.

Interpretation of the results from measuring GI can, moreover, be affected by the large day-to-day within-subject variation CV values (~25%) in normal subjects (Wolever *et al.*, 1985; Campbell *et al.*, 2003). Even anxiety/ stress evoked by the testing may influence glycaemic responses via delayed gastric emptying or release of stress hormones (Brouns *et al.*, 2005). Brouns (Brouns *et al.*, 2005) summarized the effects of other factors on glycaemic responses, as well as recommendations on how these factors could be dealt with. These factors include caffeine intake, which can decrease insulin sensitivity in humans; alcohol consumption, which in the fasting state may exert profound effects on glucose homeostasis; cigarette smoking, which can cause insulin resistance and increased cortisol secretion; acute physical exercise, which can improve insulin sensitivity on the following day; length of the overnight fast; and as previously reported the composition of the meal the evening before. Nonetheless, Campbell (Campbell *et al.*, 2003) observed that when controlling for subjects' physical activity, fasting length, and meal the evening before, previous to a standard breakfast, within-subject variability of BG response did not improve (uncontrolled trials: CV 20.4%; controlled trials: CV 24.3%). Therefore, Brouns

(Brouns *et al.*, 2005) suggested that there is no need for strict control of these factors when GI testing is concerned, except for unusual (for the subject) dietary or activity patters, and smoking, which should be avoided on the day of the testing. In the present PhD thesis, the previous mentioned factors were standardized between subjects.

2.4 Study Hypothesis/ Rationale

The overall aim of the present PhD project was to investigate whether in real-life settings (i.e. school environment), following an overnight fast, breakfast meals differing in their GI and GL produce differences in CF and mood; no studies on the combined effects of GI and GL on either measure have been reported to date. The present project focused on adolescents aged 11-14 years, as similar previous work focused on younger children. It could be argued that a low GI meal would minimize glycaemia fluctuations and facilitate performance and mood for longer following breakfast consumption compared with a high GI meal; and that a high GL would potentiate the glycaemic potency of the meal. Therefore, it was hypothesized that 1.5 hours after breakfast, high GL meals would be associated with improved cognitive performance compared with low GL meals, and that low GI meals would be associated with improved CF compared with high GI meals. As similar research suggests that different cognitive domains might be affected differently by GI, extra care was taken not only to include CF tests that have been shown to be sensitive to glucose administration and cognitively demanding, but also a variety of different measures to explore how GI and GL (low *vs.* high) affect CF and mood. Therefore, the following CF tests were used (for a detailed description of the tests see section 3.1.4.e, page 76): a word generation task, an immediate and delayed word recall task, a Stroop task, a matrices task, a speed of information processing task, and a serial sevens task. Furthermore, important confounding factors, such as iron status, social class, gender, age, height, weight, nutritional status, perceived difficulty and mood, BG and cortisol levels, and the habitual breakfast eating habits of the participants were taken into account (see section 2.1.4, page 35), and the timing of all procedures was standardized between subjects.

Three studies were conducted in order to test the previously stated hypothesis; a cross-sectional study in adolescent children, a clinical study in young adults, and an intervention study in adolescent children. The aim of the cross-sectional study, which was in fact a pilot study, was to investigate whether the glycaemic potency of breakfast affects CF and mood 90 minutes after breakfast in adolescents who have breakfast habitually (at least once per week). Blood glucose levels were measured immediately before the CF tests, to investigate whether pre-task levels correlated with performance, and immediately after the CF tests, to see if the change in BG levels during the testing correlated with performance. Haemoglobin (Hb) was also measured, as a crude measure of iron status. It would be unethical to measure these biochemical markers in venous blood sample as part of this type of research, where there is no direct benefit to the participants or an underlying disease/ condition. As such, the best available technique for use in the field was capillary (finger prick) blood sampling, using portable meters. These portable meters are included in the *in vitro* diagnostic medical devices (IVDs), which are used for the '*in vitro* diagnostic examination of specimens derived from the human body' (MHRA website). Cross-sectional studies are not as robust as intervention studies, but can provide an indication of associations that may be worth further exploration. They may also highlight potential confounders for further consideration. Indeed, the present study provided an indication that there were differences in CF and mood between meals differing in their GI and GL in a 2x2 grid, warranting therefore further investigation.

In order to test meals that were different in their GI and GL in an intervention setting, it was necessary to ensure that the meals selected differed in their glycaemic and insulinaemic responses. Therefore, the aim of the clinical study was to assess the impact of breakfast meals differing in their GI and GL on blood glucose and insulin responses over a period of three hours after the ingestion of the meals (every 15 min during the first hour, and then every 30 min); and to investigate the validity of the methods for calculating GI and GL by measuring the iAUC at 120 min. Cortisol was also measured to investigate whether the testing procedure could induce changes in cortisol levels (i.e. biological marker of stress), or even if the meals could bring about changes in cortisol levels. Since no children could be used as subjects due to the invasive nature of the study, healthy young adults were selected. The foods selected to design the breakfast meals were based on what the children reported eating in the

cross-sectional study, as well as the GI and GL of the meals, to ensure that these resembled the natural breakfast eating habits of the participants. This study allowed us to detect whether there were indeed differences in the glycaemic and insulinaemic responses of the designed meals.

In the final study, the breakfast meals that differed in their glycaemic and insulinaemic responses (i.e. produced mainly by differences in GL) were administered in adolescent school children aged 11-14 using an intervention rather than a cross-sectional study. The aim of this study was to assess in a more vigorous way than the cross-sectional study whether there exists a causal relationship between the consumption of breakfast meals differing in GI and GL on CF and mood. The mechanisms that could be mediating any existing effects were elucidated by measuring BG and cortisol, the main possible mediators as suggested by the existing literature (see sections 2.2.3 and 2.3.3, pages 50 and 60, respectively). In the field, salivary collection is the only available technique to measure cortisol, and it gives the advantage of being able to study participants in their natural environment. Furthermore, it is easy to collect, relative inexpensive, and due to the non-invasive nature of the technique, any stress induced effects should be minimal.

The anticipated benefit of the final study was to identify clearly the macronutrient composition of breakfast that could have a positive effect on the cognition and mood of adolescent school children. This would be important because it could influence policies concerning breakfast provision at school and public health attitudes to breakfast.

CHAPTER 3: SUBJECTS AND METHODS

3.1 Cross-sectional study in school children

3.1.1 Study design

3.1.1.a Summary

A cross-sectional study was carried out in two mixed secondary South London schools to test the relationship between the glycaemic potency of the breakfast consumed and CF in adolescents aged 11-14 years. Potential confounding factors such as iron status, BG levels and mood at the time of the CF tests, as well as individual effort and task demand were taken into account. Sixty pupils took part, 36 girls and 24 boys, all of whom were in good health, free from learning disabilities, and had breakfast at least once a week.

3.1.1.b Ethical approval

The study was approved by the King's College's Research Ethics Committee (reference no: 04/05-51, March 2005). Written consent was obtained from each participant and their parents/ guardians, as well as by the headteachers of the participating schools. No monetary or other incentive was given.

3.1.1.c Researchers involved

In addition to myself, two MSc students were involved in the fieldwork and data entry. The researchers were sufficiently trained and observed in the field. Criminal Records Bureau (CRB) clearance was obtained for all the researchers involved in this study, before they were to test any children.

3.1.1.d Power calculations

Power calculations were carried out using a two-sample t-test (Minitab). In order to achieve 80% power to demonstrate differences of 11.03 in the word generation (verbal fluency) task significant at the 5% level assuming a value of sigma of 15 (Donohoe & Benton, 1999a), thirty children were required for each group; where

group 1 was the low GI; and group 2 was the high GI. So, in total 60 pupils were needed to investigate differences in GI. In the final design of the study, participants were distributed into four GI and GL groups. In the analysis, however, there were always 30 students per group in either the low GI-high GI contrast or the low GL-high GL contrast.

3.1.2 Selection of schools

The schools that were invited to participate in the cross-sectional study were selected by examining the school's OFSTED (Office for Standards in Education) report. Two schools in South London, Sacred Heart R.C. School and Dunraven School were selected. Both schools were mixed comprehensives, with a wide social class and ability mix, and were close to the researchers' Department. Potential schools were approached by sending a letter (Appendix II.1) to the headteacher, explaining the purpose and nature of the study, and inviting the school to take part in the proposed study. Meetings with the schools' headteachers took place in April 2005 (Sacred Heart R.C. School) and in June 2005 (Dunraven School). The purpose, structure and timetable of the study was explained in detail at the meetings, as well as the level of support, commitment and the facilities that would be required in order to conduct the study. Both schools agreed to participate in the study.

3.1.3 Recruitment – Screening

The key safeguards relating to research involving healthy children is the provision of proper information to both participants and their parents/ guardians about the purposes, benefits and risks, and making clear to children and their parents/ guardians that the research is voluntary and that they can withdraw at any time. Participants were recruited by giving out a presentation in the year 7 and 8 assembly of the first school explaining in simple language the purposes of the study, and what exactly it would involve on their behalf. Envelopes with relevant information were distributed to the children on that day (250 in total). These envelopes included all the relevant papers for both the parents (letter, screening and consent form; Appendix II.2) and the participants (information sheet, consent form; Appendix II.3). The response rate from

Sacred Heart school was low. Therefore, 450 letters were distributed via school register at Dunraven School, eight weeks after the commencement of the study.

Students that returned the screening and consent forms, signed by both themselves and their parents or guardians were eligible to take part in the study. Each student was given a unique identification number (ID), and the screening form that his/ her parents filled in was used to exclude students on the basis of medical or other grounds. Children were selected on the basis that they had breakfast at least once a week. In order to ensure the required distribution of high and low GI breakfasts, and that not all children had the same breakfast, parents/ guardians were asked to complete in the screening form their child's typical breakfast meal; including the time they have it, as well as the type and the amount in household measures of the foods that consist the usual breakfast meal. If the main source of CHO-containing food was of low GI then the meal was classified as low GI; if on the other hand it was of high GI, then it was classified as high GI. The children, if selected, would be asked to consume this typical breakfast meal on the morning of their appointment and at the time stated in their parents' screening form.

The exclusion criteria included blood disorders or other causes of anaemia, diabetes, acute or chronic illnesses or diseases, and colour blindness. The reason for the latter is that one of the CF tests required the participants to be able to distinguish between colours. Potential participants were further excluded on the basis of severe learning disabilities and mood disorders; this information was obtained from the school in strict confidence. All the children that consented to take part but were not selected, were informed by the school for their not participation in the study.

In the first school, 63 students replied, out of whom six did not give consent, eleven were excluded and seven dropped out (n=39). In the second school, within the given time, 80 students replied who all consented; 21 were selected at random after excluding five. A total of 60 students participated. Therefore, sixteen students were excluded from both schools (nine were boys and seven were girls); out of those six never had breakfast, four were statemented (severe learning disability), one had traits of thalassaemia, two had sickle cell anaemia, one had the G6PD (Glucose-6-Phosphate Dehydrogenase) deficiency and two were colour blind. Furthermore, for

the majority of the excluded children the screening form was not fully completed; their parents were not contacted to request missing information as that would impose a burden on the families, especially since their children were excluded. Therefore, the socio-demographic characteristics of the children that were excluded are not available. Similarly, the parents that did not consent for their children to take part did not provide any information on socio-demographic characteristics (i.e. the screening form was not completed).

3.1.4 Procedure

3.1.4.a Summary

At each school, three rooms were allocated for the purposes of the study. Three researchers carried out the study, and 20 pupils on average were seen by each researcher, allowing for three appointments to be made every day. The researchers were trained on the finger pricking, the administration of the CF tests, and were all instructed to follow the same administration protocol (Appendix II.4).

An appointment was made the day before testing, by placing an appointment form in the class register (Appendix II.5). Each participant was seen once, and the time of the appointment was fixed for all participants, at 08:15. Each child was asked to have their usual breakfast on the morning of their appointment at the time they usually have it, and nothing else, with the exception of water. Children were asked to fill in in their appointment form the start and finish time of breakfast. The aim was to test children 90 minutes after the start of breakfast, to ensure that BG levels would have reached their peak values and would be either rising (having dropped below the baseline as a result of previous insulin secretion and returning to baseline – low GL breakfast meals) or falling (having reached their peak values and returning to baseline – high GL breakfast meals). Nonetheless, although children were supposed to have their breakfast at a time which was stated at their parents' screening form, this was not always the case. So, the aim was to see children as soon as possible after the 90 minutes interval, but not after 120 minutes.

On the day of the appointment the first thing that the child was asked was whether they had eaten their usual breakfast, and if they had eaten anything else apart from that. In case they had eaten something else, time of consumption was noted and an acceptance rule was used, using the Exchange lists for meal planning (Wheeler, 2003). If a participant had eaten a 'snack' as well, he/ she was either seen at the time of the original appointment, or 90 minutes after the start of the snack if it contained more than 10g of CHO. It was assumed that an intake of 10g CHO or less would not be associated with a significant change in BG levels. This was to ensure that each child would be seen 90 minutes after his/ her last high CHO food.

The order of procedures was as follows: anthropometric measurements (height and weight), the first finger prick blood sample followed by the administration of the mood scales ('before'), the CF tests, the mood scales ('after'), the second finger prick blood sample, and finally the task demand questions and the interview. All measurements and tests were recorded in the researcher's booklet (Appendix II.6). The mood scales, CF tests, and task demand questions are presented in Appendix II.7. The whole procedure lasted approximately one hour with each child, and at the end of the session each child was given a certificate of attendance (Appendix II.10).

3.1.4.b Anthropometric measurements

Height (ht) and weight (wt) were measured in all participating students, since they give the simplest measures of nutritional status (raw measurements), and they are easy to collect. Pupils were weighed in their school uniform, after being instructed to remove their blazer and shoes, on a portable weighing scale (Salter) and measured for height using a portable stadiometer (Leicester height measure, Chasmores Ltd.). Scales were calibrated regularly using standard weights. Weight is optimally measured on a platform (beam balance) scales, but are not sufficiently portable for many epidemiological settings including the current one. Bathroom scales were used to measure weight, as they are easily portable. Each measurement was taken three times and recorded in the researcher's booklet; the average of the first two measurements was used, unless they differed by more than 0.1kg (for weight) or 0.5cm (for height), in which case a third measure was taken and the two closest values were averaged. These acceptable targets for anthropometric assessment have been proposed by Zerfas (Zerfas, 1985), using a repeat-measures protocol.

3.1.4.c Physiological measurements

❖ **Capillary sampling**

In order to take a capillary blood sample to measure either BG or Hb, the most preferable puncture site is the finger, excluding the thumb and index finger, which are primarily used for gripping (NHS, 2004a). The equipment used in order to perform a finger prick were the following: alcohol wipe (mediwipe), disposable lancets (self-contained units) (Accu-check, Safe-T Pro plus), gloves, adhesive bandage, yellow bin bag, and a sharps disposal box (cin bin). All researchers followed the CDC (Centers for Disease Control and Prevention) capillary blood sampling protocol (CDC, 1997). Finger prick blood samples were carried out in duplicate, immediately before and after the CF tests and mood scales. If more than one glucose meters were used on a given day, researchers made sure that the same meter was used for the same subject. The subject was seated and the puncture site to be used was selected; usually the hand that the child did not write with, to avoid any discomfort during the CF testing. The hand was lowered below the level of the heart, and the researcher spent 5-10 sec gently massaging the hand from wrist to puncture site to increase the blood flow and to warm the hand, if it was too cold. Then the finger was cleaned with a mediwipe swipe and allowed to dry. The lancets were used to prick the side of the finger; the first drop of blood was discarded, as it contains tissue fluids which could produce inaccurate results. The puncture site was held downward and pressure was gently applied to the surrounding tissue to enhance blood flow. Strong repetitive pressure ('milking') was avoided as it could cause haemolysis or contamination of the specimen with tissue fluid. Once the required amount of blood was used to measure BG (glucose meter and strips) and Hb (Hb analyzer and cuvettes) a bandage was applied, where appropriate. All used materials were disposed off accordingly to avoid needlestick injury and risk of cross-contamination. At the end of each session the area and apparatus were thoroughly cleaned with antiseptic.

❖ **Capillary blood glucose**

Blood glucose was measured first then Hb. At each time-point, at least two repeated measurements of BG were taken, and if the readings differed by >0.2mmol/L, then subsequent readings were taken until any two readings differed by ≤0.2mmol/L; the two closest values were averaged. Besides, for subsequent readings the difference

should not be more than 5% (CV), if the same glucose meter is being used, which is the acceptable criterion of imprecision at all concentration levels (ADA, 1996). Glucose FreeStyle Mini meter (Abbott Laboratories 2005) was used to measure BG; it is a pre-calibrated 'plasma calibrated' meter that requires 0.3µl of whole blood, which is drawn into the strip by a 'capillary fill' mechanism. It covers a measurement range of 1.1-27.8mmol/L (MDA, 2004).

The working principles of BG meters used for the *in vitro* measurement of BG are explained in Appendix II.11. The FreeStyle System uses a technology based on coulometric 'non-wipe' measurement. The measurement time is on average 7 sec. The FreeStyle test strip uses the enzyme glucose dehydrogenase and coenzyme pyrroloquinoline quinone (PQQ). The manufacturer claims that because the enzyme used is oxygen-independent, unlike strips using a glucose oxidase enzyme, it is unaffected by HCT (covers an HCT range 0-60%) and oxygen levels. The osmium-based coenzyme allows glucose reaction to occur with very low applied potential or voltage between the electrodes. As such, it is also claimed that unlike strips that use iron-based mediators, the FreeStyle strip is unaffected by common interfering substances like uric acid, aspirin and acetaminophen.

Based on the MDA (Medical Devices Agency) evaluation report, the FreeStyle meter meets the acceptable criterion for imprecision, which is <5% (CV) at all concentration levels (ADA, 1996); at glucose concentrations of 3.9, 11.4, 21.1 and 26.2mmol/L the CV is 3.1, 2.7, 1.9 and 2.6, respectively. The total error of 9.9, 9.9, 9.6 and 9.9% respectively, meets the criterion for acceptable total error of no more than 10% at all levels (ADA, 1996). So, this means that the FreeStyle meter is clinically acceptable for extra-laboratory use for use with capillary finger prick samples. The quoted HCT range of 0 to 60% seems to be in reasonable agreement with that stated in the evaluation report.

The Clinical guidelines for capillary BG monitoring (NHS, 2004b) and the Safety Notice by MDA (MDA, 1996) emphasize the importance of performing quality control checks, to ensure proper functionality of the meter and test strips, and satisfactory performance of the operator. The full quality control procedure should be performed once every 24 hours, and every time a new bottle of strips is opened, or if

the subject result is unexpected. All researchers followed the above guidelines. The results from the quality control check, as well as the date a new vial was opened were recorded in the quality control logbook. The meter and strips were stored according to the manufacturer's specifications.

- **Capillary Haemoglobin**

Whole blood Hb was measured using the 'HemoCue 201^{+}' (HemoCue LtD, 2005) as a crude measure of iron status. It is a portable instrument used to measure Hb. The device has a measurement range of 0-25.6g/dL (0-256g/L or 0-15.9mmol/L), and it is particularly useful for field use. An evaluation of this device (von Schenck *et al.*, 1986) showed that the results from HemoCue are not only comparable to the 'gold standard' method (ICSH, 1978) (correlation coefficient r=0.96), but also superior in some ways. The HemoCue technique is based on the use of an optical measuring cuvette, containing dry reagents. The reaction in the cuvette is a modified azidemethemoglobin reaction. The reagents are sodium desoxycholate to disintegrate the erythrocytes, sodium nitrite to oxidize Hb to methemoglobin, and sodium azide to form azide methemoglobin. A small blood sample (10µl) is drawn up into the cuvette by capillary action where it is mixed with the dry reagents. The cuvette is then placed in the HemoCue photometer, which uses a double wavelength measuring method, 570nm and 880nm, for compensation of turbidity. A reading is given out within 60 sec. The advantages of the method are that imprecision associated with dilutions and sampling is avoided, and turbidity from protein, cell stroma and lipids does not elevate the reading. The reproducibility (CV) of the HemoCue analyzer using duplicate samples is 1.3%. The meter and the microcuvettes were handled and stored according to the manufacturer's guidelines. Quality control checks were performed according to the manufacturer's recommendations.

3.1.4.d Mood scales

A self-rating questionnaire was developed from the Profile of Mood States bipolar form (POMS-BI) (Lorr & McNair, 1988) and the short form of the Activation-Deactivation Adjective Checklist (AD ACL). It was modified from previous research (Rogers *et al.*, 1995) for the purposes of this study, in order to develop a format that is appropriate for this age group. It has been found to be useful in students of this age

group and nutrition studies (Nelson *et al.*, 1994; Nelson, 1996; Nelson *et al.*, 2001). Participants were presented with a list of 22 words (Appendix II.7) used to assess mood, energy levels, hunger and thirst, and they were asked to rate each word according to how they were feeling at that moment; once before the administration of the CF tests, and once immediately after. Responses were made on integer scales from zero ('not at all') to four ('extremely'). In case the subject was not sure of a certain word or did not know how to interpret it, then a synonym was provided. A list of the same synonyms for the same word was given out to all the researchers, so that the same explanation was provided to all participants (Appendix II.8).

3.1.4.e Cognitive function tests

The CF tests that were used in this study (Appendix II.7) have been used in nutrition studies, in order to detect differences of a nutrient intake on cognitive ability (see sections 2.2.2 and 2.3.3, pages 42 and 60). Furthermore, they have proven to be sensitive in detecting differences in CF induced by the administration of glucose (Donohoe & Benton, 1999a; Kennedy & Scholey, 2000; Sunram-Lea *et al.*, 2001). Cognitive function tests were thoroughly explained to the participants, and a small training session before each test was also administered to ensure that each subject had understood what the test involved. No participant was allowed to ask any questions during the tests. If there was any indication that the participant had not understood what the test involved, even after the test had started, then the test was stopped, and the participant was reinstructed. Tests were timed accurately using a stopwatch. For the tests that required the participant to say something out loud responses were both written down by the researcher and recorded with a tape recorder. The tests were administered in the same order for each participant, as follows:

- **Word generation task (word memory – verbal fluency, working memory)**

Participants were asked to generate out loud as many words as they could beginning with the letter 'S' (excluding names of people or places); they were given two minutes to complete this task. This verbal fluency task 'assesses access to and retrieval of information stored in long-term memory' (Halpern, 1992), as used by Scholey (Scholey *et al.*, 2001). The test was scored for the number of words that are recognizable and begin with the letter 'S' (unique with different roots), which was the

main variable; and for the number of words that were generated incorrectly, either starting with a different letter or just did not make any sense.

❖ **Immediate and delayed memory (word list, short- and long-term memory)**

The same list of 15 random words (e.g. product, deed, hide, and glory) was presented to the participants for 45 seconds (three seconds per word). The words were all nouns between four and seven letters long, and they were all of similar frequency and imagery (Appendix II.9). No plurals or names were used. The participants were told that would be required to remember as many words as possible. Immediately after the presentation of the words, then again 25 minutes later (last test), participants were given two minutes in which to write down as many words from the list as they could remember. The list of words was adapted from Heatherley (Heatherley *et al.*, 2005) using the word list generator website http://www.math.yorku.ca/SCS/Online/paivio/. For both immediate and delayed recall, scores were based on the total number of words recalled correctly (main variable). Items generated that were not on the list were also recorded.

❖ **Stroop task – colour-word 'interference' task (selective attention, vigilance)**

The Stroop task (Stroop, 1935; Stroop, 1938) has been used and adapted from studies examining the effects of glucose administration on CF (Benton *et al.*, 1994). This task involved naming the colour of the ink in which incongruent colour names were printed (e.g., the word 'red' printed in green ink). Each subject completed two tasks: a 'control' condition, where rows of Xs the same number of letters as the name of a colour were presented in that colour; and an incongruent condition ('actual' test) were the six colour names were presented as words written in conflicting colours. The colours that were used were white, black, blue, red, green, yellow, which were displayed in a grey background (a total of 60 words in either condition). Participants were asked to say out loud the colour of the ink, as quickly and as accurately as they could, moving across each row from left to right. This task tests vigilance (also known as attention), rapid information processing and flexibility. The subject must attend to certain features of stimuli and ignore others; function which is referred to as response inhibition (Dye *et al.*, 2000). The time taken to complete the task (for both conditions) was recorded; the time to complete the 'actual' test and the 'interference' score were used as the main scoring variables. The 'interference' score was computed as the time

of the 'actual' test minus the time of the 'control' test; the latter score implies that the less the 'interference' the better the cognition. Accuracy of the responses ('correct' responses and 'errors') was also recorded.

❖ **Matrices (non-verbal IQ, general fluid intelligence)**

This task was adapted from the British Ability Scales II (Elliot *et al.*, 1996) and is thought to assess inductive reasoning (non-verbal IQ). It required participants to select a shape or arrangement of shapes that completed a sequence of similar shapes. Sixteen of these problems (matrices) were presented in order of increasing difficulty. The number of matrices completed correctly within six minutes was recorded (key variable).

❖ **Speed of information processing – Number search task (sustained attention, vigilance, working memory)**

This speed of information and selective attention task was adapted from previously published methods carried out by Manly (Manly *et al.*, 2001), and Heatherley (Heatherley *et al.*, 2005). The participants were presented with a single A4 page on which was printed a total of 1,280 single or double digit even and odd numbers. They were asked to circle blocks of three consecutive odd numbers, working from left to right and row by row as quickly and as accurately as they could. Three minutes were allowed to complete the task. The test was scored for the number of hits (three consecutive odd numbers correctly identified – main variable), 'misses' and 'errors'.

❖ **Serial sevens (working memory, vigilance)**

Serial sevens involves presenting a participant with a starting number from which they must subtract seven, then subtract seven from that number and so on. The starting number, 857, was the same for all participants. This subtraction task was originally designed by Hayman (Hayman, 1942), and appears to be sensitive to both lowered (Taylor & Rachman, 1988) and raised (Kennedy & Scholey, 2000) BG levels. Three minutes were given for participants to subtract the value seven, in sequence, and to say the answers out loud, as quickly and as accurately as possible; the responses were recorded on both tape and paper. The task was scored for both total number of 'correct' subtractions (main variable) and number of 'errors'. In case

of a subtraction 'error', subsequent responses were scored as 'correct' if they were 'correct' in relation to the new number.

3.1.4.f Task demand questionnaire

On completion of the test battery, the mood scales were carried out for the second time, to observe whether anything had changed in the reported mood of the participants. Immediately after the mood scales, in a self-reported task demand questionnaire participants rated how difficult, effortful and tiring they found the tests to be (Appendix II.7). A rating scale similar to the mood scales was used.

3.1.4.g Interview questions

Each participant was interviewed by one of the researchers following the second finger prick. Questions were asked about the participant's eating habits, physical activity, current health status, medication/ supplements (if any), body image perception, sleeping patterns, and menstrual status (girls only) (Appendix II.6). These questions also enquired in detail about the food and drink consumed at home on the morning of the testing ('breakfast'); anything else eaten or drank on their way to school or since arriving at school ('snack'); and the last meal they had the night before their appointment ('dinner').

The dietary intake methods that were used in the PhD studies were based on retrospective dietary assessment methods, rather than prospective. A multipass approach was used in all dietary assessment methods (Moshfegh et al., 2008): that is information was first collected on what the participants had to eat (i.e. a list of foods and drinks consumed); then on the amount of food and drinks consumed; and finally, participants were prompted to remember any other foods and drinks consumed. The aim of the selected dietary assessment methods was to estimate GI and GL, and not dietary patterns or daily energy consumption. To determine relations of the glycaemic potency of breakfast with cognitive function, the available CHO intake must be ascertained, in order to calculate meal GI and GL. Estimating the intake of available CHO using diet records or questionnaires can be challenging, in that it requires separate estimation of each of the following: (a) the quantity or portion size of each

food item consumed, (b) the precise available CHO content of each item, and (c) the cooking methods used in food preparation.

Estimation of the quantity or portion size of each food item consumed was performed by using a Food Atlas (Nelson et al., 1997). The use of food photographs to estimate dietary intake of breakfast and dinner can be challenging, considering that in general small portion sizes tend to be overestimated and large portion sizes to be underestimated. Nonetheless, the Food Atlas has been shown to provide an objective measure of exposure for breakfast, lunch and dinner (Nelson *et al.*, 1996). The use of food photographs as an appropriate method for dietary recall and portion size assessment has been validated for use in young adults (Nelson *et al.*, 1996) and it was felt appropriate for use in children aged 11-14. (The Atlas has since been used successfully in a study of low income households, including adolescents (Nelson et al., 2007).) The correlation of the nutrient content of meals based on the actual or the estimated portion size ranges from 0.84 to 0.96. In fact, misclassification of subjects according to their nutrient intake is reduced when using this method of dietary intake rather that using average portions. Besides, using photographs has many advantages: (1) photographs can be easily copied/ carried and incorporated into food questionnaires; (2) they include a wide range of foods and appropriate substitutes, which makes them highly specific; and (3) can be easily posted making them appropriate for large scale epidemiological studies.

Three main functions are needed on a subject's behalf to assess a food portion size when using the Food Atlas: perception ('the ability to relate an amount of food which is present in reality to an amount depicted in a photograph'); conceptualization ('the ability to make a mental construct of an amount of food which is not present in reality, and to relate that to a photograph'); and memory ('which will affect the precision of the conceptualization') (Nelson *et al.*, 1996). The use of the specific Food Atlas, which uses eight photographs to assess portion size for an individual food is associated with small errors in the perception and conceptualization of portion size. Memory is a function that is affected by individual ability, and is expected to affect more past recalls (i.e. dinner) compared with more recent ones (i.e. breakfast). Nonetheless, the Food Atlas appears to be the most appropriate and reliable choice when evaluating dietary intake in epidemiological settings where it is not possible to

weigh the food eaten (i.e. retrospective vs. prospective dietary assessment methods). The errors associated with estimates of portion sizes are reflected in the estimates of the nutrient content of the meals to which these foods contribute. The average errors associated with the use of the specific Food Atlas are within ±7% of the calculated energy and nutrient contents of the meals based on actual portion sizes, which is considered acceptable when estimating the nutrient intake of groups of people in cross-sectional settings. Photographs improve estimates of the nutrient content of meals and reduce subject misclassification. Imperfect estimation of dietary intake would bias results towards the null.

Each of the eight photographs in each set was A7 in size (approximately 75x100 mm, landscape) and showed one portion of food on a plate or in a bowl with either a knife and fork or dessertspoon (respectively) in view, photographed against a plain white background. The smallest and largest portion sizes depicted in the photographs represented the 5th and 95th centiles of consumption for that food as recorded in the NDNS adult survey (Gregory et al., 1990). The six remaining photographs depicted portion sizes at equal weight intervals between the smallest and largest portion sizes. The nutrient content of the meals were estimated from food composition tables (see section 3.1.5.c). Where the exact food was not depicted, a food of similar texture and appearance was used. When specific prepackaged products had been consumed, such as crisps, and biscuits, the weight of the product was found by identifying the item in local shops and noting the weight on the packet.

3.1.5 Data analysis

3.1.5.a Glycaemic Index and Glycaemic Load

The GI value of the individual foods that comprised the three different meals (breakfast, snacks, and dinner) was derived from the 'International table of glycaemic index and glycaemic load values' (Foster-Powell *et al.*, 2002), and from a more recent publication referencing GI and GL values of commercially available products in the UK (Henry *et al.*, 2005). Where several entries were available for the same food, a preference was given to the GI value from a European food; where a food consumed could not be found, the GI value of an entry closest to the type and macronutrient

composition of the food consumed was used. The GI of the composite meals was calculated as the sum of weighted GI values of the foods comprising the meal, and the GL of the composite meal as the sum of the GL values of all the foods comprising the meal (see section 2.3.2, page 59).

3.1.5.b Socio-occupational classification

The screening form filled in by the parents/ guardians (Appendix II.2) was used to collect information with regard to the parents'/ guardians' occupation, status in employment and industry, and size of employing establishment. These information were used to classify people in socio-economic groups (SEG) (1–17) based on their occupation (Government Statistical Service, 1990); the SEG was defined according to the highest of either the father or the mother. The non-employed (i.e. retired, homemakers, sick, disabled), and full-time students were classified by their last main job. For analysis of variance (ANOVA), SEG was categorized into three larger groupings: 'professional and managerial' (SEGs 1, 2, 3, and 4; n=23); 'skilled and technical' (SEGs 5, 6, 7, 8, and 9; n=15); and 'semi-manual' (SEG 10; n=22).

3.1.5.c Nutrient analysis

Microdiet (Downlee Systems Ltd.) was used to analyze the nutrient content of the foods consumed for breakfast, snacks, and dinner. A file was generated for each participant including the three meals. Nutrient data was input to SPSS and merged with other subject variables; the latter were created by coding the data from all questions and answers in the booklets used. The UK nutrient database supplied with Microdiet includes the most recent UK food composition tables by McCance and Widdowson's (6^{th} edition) (FSA, 2002).

3.1.5.d Physical activity

Physical activity (PA) can be assessed by using both objective and subjective measures (Melanson, Jr. & Freedson, 1996; Rennie & Wareham, 1998). The aim of the present study was not to estimate daily energy expenditure or energy cost from specific activities; merely to examine whether exercise the day before or on the morning of testing may have an effect on the performance of the participants. Self-reported PA is a subjective measure, and three subjective questions were used to assess leisure activity levels: duration, intensity, and frequency (where applicable). The questionnaire used was based on the one used for the National Diet and Nutrition survey (Gregory *et al.*, 2000). The Compendium of Physical Activities (Ainsworth *et al.*, 1993; Ainsworth *et al.*, 2000) was used to assign a MET (respective metabolic equivalent intensity level) score to each one of the activities, based on the reported intensity. MET is defined as 'the ratio of work metabolic rate to a standard testing metabolic rate of 1.0 (4.184 kJ) x kg^{-1} x h^{-1}; 1 MET is considered as resting metabolic rate obtained during quiet sitting'. A PA score was allocated to each one of the activities (MET score x duration in hours), and the PA scores of all activities were added to obtain the total PA score of the day before the appointment, and of the morning of the appointment. Based on the total PA scores, participants were distributed into thirds of the distribution of PA for each one of the variables under interest; light, moderate and vigorous.

3.1.6 Statistical analysis

Statistical analysis was conducted using the SPSS 13.0 (Statistical Package for the Social Sciences). Differences in anthropometry, nutrient content of breakfast, and CF test results between the four GI – GL groups were assessed using one way analysis of variance (ANOVA). Influences of potential confounders were assessed using correlation and multiple regression analysis. Further ANOVA was carried out for each one of the main CF test scores as dependent variables with main factors and covariates to determine the final model for each one of the tests.

3.2 Predicting glycaemic and insulinaemic responses from mixed breakfast meals

3.2.1 Study design

3.2.1.a Summary

An intervention trial was carried out to investigate the postprandial responses over a period of three hours after the ingestion of five breakfast meals, differing in their GI and GL; and to investigate the validity of methods for calculating GI and GL by measuring the iAUC (glucose and insulin). Ten young adults (five males, five females, age 18-30 years) received all breakfast meals in randomized order. Capillary and venous blood samples were collected at baseline, and then at 15, 30, 45, 60, 90, 120, 150, and 180 min after breakfast. Blood glucose, insulin and cortisol were measured at each one of the time-points. All participants were in good health, and had breakfast at least twice or more a week.

3.2.1.b Ethical approval

The study was approved by the King's College's Research Ethics Committee (reference no: 05/06-25, 15th December 2005). Written consent was obtained from each participant. Participants were compensated for their time with a small token of appreciation on completion of the study (£75).

3.2.1.c Researchers

One undergraduate student assisted in the fieldwork, after being sufficiently trained in most aspects of the administration protocol (Appendix III.1). I received formal training in venepuncture and cannulation (qualified phlebotomist), so as to perform this aspect of the administration protocol.

3.2.1.d Power calculations

The number of participants recruited were based on the standard GI testing protocol, where at least eight subjects have to receive the same test meal (FAO/WHO, 1998; Wolever *et al.*, 2003).

3.2.2 Recruitment – Screening

Following receipt of ethical approval, a circular e-mail was sent to all students and staff at King's College London (Appendix III.2). People that responded and expressed interest in taking part, further received an information sheet (Appendix III.3) and a screening form (Appendix III.4). The screening form was completed by all potential participants, in order to exclude subjects for medical or other reasons. The breakfast eating habits of the participants and the type of breakfast typically consumed were also recorded. The exclusion criteria are listed here:

- Age <18 and >30 years
- Sickle-cell anaemia, Haemophilia, Thalassaemia traits, or any other blood disorders
- Diabetes or other glucose tolerance disorders
- Any other chronic diseases
- Medication received on a regular basis that affects glucose or cortisol metabolism
- Allergy or intolerance to any of the components of the breakfast meals (e.g. milk, nut, wheat)
- Breakfast omitters (i.e. never have breakfast)
- Underweight or obese (BMI <18.5kg/m^2 or >30kg/m^2) (WHO, 2000)
- Professional athletes or subjects taking part in exhaustive training
- Heavy smoking (>4 cigarettes a day)

Therefore, a participant was eligible to take part if the above criteria were met. Furthermore, if a participant had any infections or colds prior to his/ her participation, the appointment was postponed until he/ she felt better and the symptoms were relieved.

3.2.3 Procedure

3.2.3.a Summary

Ten young adults (five males, five females) agreed to take part out of the 176 that originally responded to the circulated e-mail (none was excluded); these ten adults also met the inclusion criteria. All ten participants completed all five visits. Informed written consent (Appendix III.5) was obtained from each one of the participants. Eligible subjects were seen on five separate occasions at the metabolic room of the Department of Nutrition and Dietetics, King's College London, one week apart; where the latter was not feasible the subjects were tested at least 2-3 days apart. Appointments were made for all five visits, and participants were given an instructions sheet (Appendix III.6) to follow the day before and on the morning of their appointment. The instructions given were based on the standard GI testing protocol (Wolever *et al.*, 2003). On the day before each of their appointments subjects were asked to eat their normal diet, to avoid smoking and alcohol consumption, to restrict consumption of caffeine containing drinks, to restrict their participation in intense physical activity (e.g. long periods at the gym), and to have a dinner of their choice that had to be the same before each appointment. Dinner before each appointment was instructed to be recorded in household measures, to be consumed in <20 minutes, and to be finished by 21:00 the latest; after 21:00 subjects were told not to eat or drink anything else, apart from water. They were also instructed to sit quietly after dinner and before bed. Subjects were studied after a 10-12 hour overnight fast. On the morning of each testing day, participants were asked to avoid any form of physical exercise and to come to their appointment at 08:00 using the least strenuous means of transport. On average, two to three subjects were seen on each day.

Upon arrival, the order of procedures was as follows:

i. Screening on the day, to ensure that the participant did not have anything to drink or eat, and they were not feeling unwell (i.e. sick)
ii. Anthropometric measurements (ht, wt); weight was measured on all five visits
iii. Baseline capillary blood sample, to measure BG and Hb
iv. Cannulation of the subject
v. Fasting venous blood sample

vi. Breakfast administration (time zero)

vii. Capillary and venous blood samples at 15, 30, 45, 60, 90, 120, 150, and 180 min after breakfast

viii. Interview

ix. Lunch was offered to all participants on completion of each testing day

All information collected and measurements taken were recorded in the researcher's booklet (Appendix III.7). The whole procedure lasted approximately four hours.

3.2.3.b Breakfast meals

The five breakfast meals were administered following the baseline venous blood sample in randomized order for all participants. Participants were blinded to the meal they would receive. The GI and the GL of the breakfast meals were based on the median GI and the GL of the reported breakfast meals from the cross-sectional study. Similarly, the foods that constituted the breakfast meals were based on what the participants reported eating in the first study (see Table 4.2 and Table 4.3). Each individual food was weighed with food scales (Precisa XB 3200D, Precisa Instruments Ltd./ Switzerland) to the nearest 0.1g (weighing range of 5–3,200g x 1g) on the morning of the testing, to ensure that the GI and GL calculated corresponded to the original formulation. Participants were instructed to consume the meal at a comfortable pace within 15 min, and to consume all food and drink provided; otherwise, they would have to be excluded from the study. Time zero was regarded as the time when eating commenced. It was then that a stopwatch for each participant was set, to ensure that all repeated capillary and venous blood measurements were taken at the exact time-points. Time taken to finish the meal was accurately timed and recorded. After breakfast, participants could not eat or drink anything else, unless they were thirsty and then water was offered, the amount of which was accurately measured and recorded. Participants were also instructed to remain sitted, and to be as calm and relaxed as possible throughout the testing period.

The macronutrient and micronutrient composition of the individual foods that consisted the breakfast meals was found from the Nutrient Databank (see section 3.3.4.c, page 111), or by contacting the manufacturer, if the food item could not be found in the Databank. The GI of the individual foods (reference food: glucose; GI glucose=100) was found either from the International Table of GI and GL values (Foster-Powell *et al.*, 2002) or from more recently published values based on UK products (Henry *et al.*, 2005). The mean GI values (±se – standard error of mean) for each one of the foods used to prepare the breakfast meals are: Alpen muesli, no added sugar (Weetabix) 55±10 (International Table, food entry: 198), Kellogg's Corn Flakes 81±3 (International Table, food entry: 168), Semi-skimmed milk (Tesco's) 25±6 (UK products, food entry: 66), Apple juice, fresh (Tesco's) 40±1 (International Table, food entry: 32), Sugar white (Tate and Lyle) 68±5 (International Table, food entry: 589). The GI of the composite meals was calculated as the sum of weighted GI values of the foods comprising the meal, and the GL of the composite meal as the sum of the GL values of all the foods comprising the meal. The total volume of the meals was made the same (500ml) by giving water. Furthermore, the meals were designed so that there was a two-fold difference in GL between the high and the low GL meals, to ensure that there was enough difference between them to detect statistically significant differences in glycaemic and insulinaemic responses.

The breakfast meals differed in their GI and GL (2x2 grid): a low GI – high GL (M1), a high – GI high GL (M2a) of similar GL to (M1), a high GI – high GL (M2b) of similar energy and macronutrient composition to (M1), a low GI – low GL (M3) and a high GI – low GL (M4). The reason why we included M2b is to be able to compare M1 to a meal of similar energy and macronutrient composition; M2a though was of similar GL, differed a lot in the energy content (20%) when compared to M1. When it came to the low GL meals, the calculations provided two meals with similar energy content and GL, so there was no need to include an extra meal. The foods that consisted the five breakfast meals used, as well as the macronutrient composition, GI and GL are presented in Table 3.1 and Table 3.2, respectively. The % macronutrient composition, as well as the composition per 100g for each one of the foods used is presented in Appendix III.8 and Appendix III.9, respectively.

Table 3.1: Foods (g) that consisted the breakfast meals administered in the intervention study in young adults.

PRODUCTS (g)	BREAKFAST MEALS				
	HIGH GL			LOW GL	
	Low GI (M1)	High GI (M2a)	High GI (M2b)	Low GI (M3)	High GI (M4)
Alpen Muesli no added sugar (Weetabix)	66	0	0	40	0
Kellog's Corn Flakes	0	48	55	0	30
Tesco's Semi-skimmed milk	200	250	300	250	300
Tesco's Apple juice	245	150	200	0	0
Sugar white	10	5	10	5	5
Volume of liquid food	445	400	500	250	300
Water	55	100	0	250	200
Total volume	500				

Table 3.2: GI, GL and macronutrient analysis of the breakfast meals administered in the intervention study in young adults.

	BREAKFAST MEALS				
	HIGH GL			LOW GL	
	Low GI (M1)	High GI (M2a)	High GI (M2b)	Low GI (M3)	High GI (M4)
GI meal	49	62	61	48	61
GL meal	44	46	57	21	28
Energy (kcal)	481.7	387.6	480.6	281.2	275.6
Energy (Kjoule)	13.9	11.8	14.0	12.5	12.0
Protein (g)	7.1	4.4	5.3	6.4	5.1
Fat (g)	2.7	2.8	3.4	3.0	3.4
• of which saturates (g)	89.6	74.5	93.4	43.2	45.2
Total CHO (g)	57.7	38.0	51.6	23.9	22.4
• of which sugar (g)	31.9	36.5	41.8	19.4	22.8
• of which starch (g)	40.0	22.8	33.5	6.7	5.7
Sugar breakdown	17.7	15.2	18.1	17.2	16.7
• NMES (g)	9.8	4.8	6.4	1.6	0.3
• Intrinsic & Milk Sugar (g)	20.3	11.0	14.6	1.8	0.3
Sugar breakdown	13.0	8.0	13.8	5.0	5.7
• Glucose (g)	0.0	0.0	0.0	0.0	0.0
• Fructose (g)	11.8	12.5	15.0	13.6	15.0
• Sucrose (g)	3.1	1.8	2.1	1.9	1.1
• Maltose (g)	5.1	1.4	1.7	3.1	0.9
• Lactose (g)	115.7	92.1	114.3	65.2	63.2

3.2.3.c Anthropometric measurements

Subjects were weighed in their clothes, after being instructed to remove any heavy clothing and shoes and to empty their pockets, on Tanita Floor Standing Body composition analyzer (Type BC-418 MA, Marsden The Weighing Company); 1kg was taken out for clothing. Participants were measured for height using a wall-mounted mechanical stadiometer (Chasmores, Ltd). Body composition analysis was also recorded. The same methodology for repeated measurements was used as in the cross-sectional study (see section 3.1.4.b, page 72).

3.2.3.d Physiological measurements

Capillary (finger prick) and venous blood samples were collected at baseline and then again at 15, 30, 45, 60, 90, 120, 150, and 180 min after breakfast. At each time-point the finger prick blood sample was taken first, followed by the venous blood sample. For each one of the time-points the following measurements were taken: capillary BG readings, using two portable glucose meters; and venous measurements of insulin, cortisol, and glucose, following biochemical analysis. Capillary and venous measurements of Hb using a portable device were taken at baseline, 60 and 180 min after breakfast. Subjects warmed their hands and arms in electrically heated blankets for 3-5 min before each blood sample.

3.2.3.d.I Capillary blood measurements

Capillary procedure was performed as explained before (see section 3.1.4.c, Capillary sampling, page 73). Blood glucose was measured first then Hb. Two different BG meters were used for the measurement of capillary BG: the Accu-Chek Aviva BG meter (Roche Laboratories 2005), which is a whole blood calibrated meter, and the Freestyle Mini meter (Abbott Laboratories 2005), which is a plasma calibrated meter (see section 3.1.4.c, Capillary BG, page 73). The reason why two different meters were used, whole blood and plasma calibrated, is to be able to compare the results between the two meters. Besides, the GI of foods is preferably measured in capillary whole blood (see section 2.3.1, page 55), and in the cross-sectional study we used the plasma calibrated one. As such, BG was always measured first with the AVIVA

meter, followed by the FreeStyle meter. Haemoglobin was measured using 'HemoCue 201+' (HemoCue Ltd, 2005) (see section 3.1.4.c, Capillary Haemoglobin, page 73). All the portable meters were used and stored according to the manufacturer's specifications.

The method for taking repeated measurements of glucose at each time-point has been described before (see section 3.1.4.c, Capillary BG, page 73). The two closest values were averaged. Participants were excluded on that day if capillary whole blood fasting glucose was ≥5.6mmol/L and <6.1mmol/L and <3.6mmol, and if plasma fasting glucose was ≥6.1mmol/L and <7.0mmol/L and <3.9mmol/L (ADA, 2006); suggesting that either the participants were glucose impaired or simply they had something to eat or drink. The lower end was set to avoid hypoglycaemia. If they insisted on having nothing to eat or drink that morning, then they were excluded on the whole from the study on the grounds of suspecting glucose impairment. This was never the case for any of the participants.

As far as the technical specifications of the Accu-Chek Aviva meter are concerned and as evaluated by the NHS Purchasing and Supply Agency (NHS PASA, 2005), this glucose meter uses non-wipe biosensor technology to produce a result in 5 sec (sample volume: 0.6µl), covers a HCT range of 20-70%, and a measurement range of 0.6-33.3mmol/L. When compared to the enzymatic reference method for glucose (hexokinase/ G6PDH method) it gave a correlation coefficient of 0.99. The Aviva test strip uses the enzyme glucose dehydrogenase and the coenzyme PQQ. According to the evaluation report, the Aviva BG meter meets the acceptable criterion of imprecision (CV<5%), at all concentration levels; at glucose concentrations of 3.7, 9.3, 19.1 and 25.0mmol/L the CV was 3.6, 2.7, 2.7 and 2.4% respectively. Total error of 4.2, 5.0, 2.8, and 2.6% meets the criterion for acceptable total error of no more than 10% at all concentration levels. This effectively proves that the AVIVA meter is clinically acceptable for extra-laboratory use. The quoted HCT range of 20-70% is in agreement with that stated in the evaluation report.

3.2.3.d.ii Venous blood measurements

The guidelines for adult venepuncture were followed in order to obtain a blood sample (NHS, 2004c). Following the fasting finger prick blood sample, subjects were cannulated (BD Venflon ™, 22 GA); the catheter was stabilized using an IV dressing (Veca-C box, BD). A fasting venous blood sample was then collected with a sterile 10ml syringe (BD). The first drop of venous blood was used to take an Hb reading from the HemoCue. The rest of the venous blood was transferred into three vacutainers (BD), in the following order: (1) serum – red colour (6ml of venous blood), (2) EDTA – purple colour (2ml of venous blood), and (3) fluoride oxalate – grey colour (3ml of venous blood) for the measurements of (1) insulin, serum ferritin (SF), serum transferrin receptor (STfR), cortisol, (2) full blood count (FBC), and (3) glucose, respectively. FBC, SF, and STfR were measured only in the fasting sample of the first and last visit for FBC, and the first visit for SF and STfR for each one of the subjects.

EDTA tubes were placed immediately in a polystyrene box with ice, and sent by courier to the Biochemistry Lab, King's College Hospital for analysis. The serum tubes were left to stand for 15-20 min, until the blood clotted. Within 30 minutes after collection, the serum and fluoride oxalate tubes were centrifuged (Jouan, Model: CR-412) at 3,000rph (2-4^{0}C) for 15-20 minutes. The serum was separated from the serum tubes using 1ml graduated pastettes (Alpha laboratories Ltd.), and the plasma from the fluoride oxalate ones. The serum from each tube was transferred into two 1ml cryovials (Alpha laboratories Ltd.); one was used for the measurement of insulin and SF, and the other one for the measurement of cortisol and STfR. The plasma from the fluoride oxalate tube was similarly transferred into a 2ml cryovial, which was used for the measurement of glucose. The cryovials were stored in cryovial boxes (Alpha laboratories Ltd.) at either –20^{0}C for one month or –80^{0}C for longer periods of time, and sent in batches to the Biochemistry Department for analysis. The methods used to measure each one of the above markers, as carried out by the Biochemistry Lab, are presented in Appendix III.10.

3.2.3.e Interview

On completion of the three hours, each subject was interviewed and the following parameters of the day before each appointment were recorded: consumption of caffeine and alcohol, the last meal, any physical exercise, smoking, time they went to bed and time of waking up. The Food Atlas (Nelson *et al.*, 1997) was used to assist participants in quantifying the amount of food and drink consumed.

3.2.4 Data analysis

The macronutrient analysis, GI and GL of the meals consumed the evening before and the physical activity levels were analyzed as presented in section 3.1.5 (page 81). Furthermore, the iAUC for both glucose and insulin (glycaemic and insulinaemic responses) was calculated as the incremental area under the response curve, ignoring the area beneath the fasting concentration. The excel formulas to calculate the iAUC was kindly provided by Dr Thomas M. S. Wolever (Department of Nutritional Sciences, University of Toronto, and Glycaemic Index Testing, Inc., Toronto, Ontario, Canada).

3.2.5 Statistical analysis

Statistical analysis was conducted using the SPSS 14.0. Repeated measures ANOVA was carried out to examine the differences in all physiological measures at each time-point between meals and in the iAUC for both glucose and insulin, using two within-subject factors, GL and GI. The influence of possible predictors on glucose and insulin iAUC was assessed using correlation and multiple regression analysis.

3.3 Intervention study in school children

3.3.1 Study design

3.3.1.a Introduction

The final part of this PhD consisted of the intervention study in school children, aged 11-14 years, to further investigate and establish whether a causal relationship exists between the GI and GL of breakfast consumed and CF in adolescents. The same protocols for CF and mood were used as in the cross-sectional study, in order for the results to be comparable. The main difference between the two studies is that in the final study the breakfast meals were administered rather than recorded. As such, four out of the five breakfast meals that were tested in young adults, a low GI – high GL (M1), a high GI – high GL (M2b), a low GI – low GL (M3) and a high GI – low GL (M4) were administered in 32 pairs (32 males, 32 females) of matched children in a cross-over design; another ten participants (five males, five females) were tested for whom an appropriate match was not found. Similar to the cross-sectional study, all participating students were in good health, free from learning disabilities, and had breakfast at least once a week. The participants had to be matched in pairs to avoid increased drop-out rates associated with asking a child to come back four times, and missing many school classes in total.

The testing of the meals in adults revealed that the high GL meals were significantly different to the low GL meals, with regard to the glucose and insulin responses; the effect of GI was less clear. Based on this finding, participants were paired based on GL rather than GI, as is was hypothesized that the GL differences would be large enough to be detected even in a matched pair; this would not be the case for the GI. As such, the study was designed so that the each child would receive either the low or the high GL breakfast, while their match the high or the low GL, respectively. Therefore, each child would have to be seen three times; once where the matching and inclusion criteria would have to be established (screening), and on two further occasions, where either the low or the high GI meal would be administered in randomized order. Potential confounding factors such as iron status, BG levels, stress (salivary cortisol) and mood at the time of the CF tests, as well as individual effort and task demand were taken into account.

3.3.1.b Ethical approval

The study was approved by the King's College's Research Ethics Committee (reference no: 04/05-105, 10th November 2006). The SFT (DfES) was an external collaborator. Written consent was first obtained by the headteachers of the participating schools, and then individually from each participant and their parents/ guardians. No monetary or other incentive was offered to the participants. The SFT offered 500 pounds to the schools on completion of the study as a token of appreciation for the staff allocated to the study, as well as for the facilities provided.

3.3.1.c Training and researchers involved

Sufficient training was provided for all the researchers involved in all the aspects of the administration protocol. Criminal Records Bureau clearance was obtained for all the researchers involved, before they were permitted to come in contact with any children. The study ran from the 27th November 2006 until the 6th July 2007. During the course of this study in addition to myself, two undergraduate students, two postgraduate (MSc) students and five research assistants were involved the fieldwork and data entry.

3.3.1.d Power calculations

For purposes of calculation, the model has repeat measures (high and low GI) within groups and contrasts between groups (high and low GL). We wanted to know the significance of the differences within and between groups, and the interaction (if any) between GI and GL (i.e. whether any observed differences between the GI repeat measures differ between the two GL groups).

The principal element of variation between CF scores was likely to be between the high and low GL groups. So, the test for power was based on un-paired t-test (for simplicity). The contrast between the high and low GI was based on paired observations within the same subjects, so the appropriate test was a paired t-test. The two sets of power calculations were likely to yield a different value for n for each contrast.

The power calculations should be based on the anticipated differences between the means from the previous cross-sectional study (see Table 4.10). Calculating power and n for very small differences will result in low power and high n, and should therefore be avoided. As such, the power calculations were based on the speed of information processing test. For the contrasts between the GL groups (high and low) the average difference between the GL groups was 2.5, and the average SD=3.2. Using Minitab 14.0 it was found that in order to achieve at least 80% power (alpha=0.05) 50 observations per group would be needed. Repeat observations within 25 subjects, however, will reduce the estimate of within group se, thus the observed power will be higher, or the number of observations required per group between 25 and 50. As far as the contrasts between GI groups (high and low) are concerned the average difference between the GI groups was 1.6, and the average score 12.2. So, the difference between any two GI groups expressed as a percentage of the mean is roughly 1.6/12.2 x 100=13%. As the results from the cross-sectional study were based on between-subject observations, the SD for within-subject observations is not known. Nonetheless, we would expect it to be similar to the SD deriving from between-subject observations, or even smaller.

3.3.2 Recruitment – Screening

3.3.2.a Selection of schools, response rates and timetable

The same schools that took part in the cross-sectional study were invited to take part in the intervention trial in September 2006 by sending a letter to the headteachers explaining the nature of the study (Appendix IV.1). Dunraven agreed to participate in the study, while Sacred Heart R.C. refused. Meetings with the school's deputy headteacher took place in November 2006, to finalize the level of commitment of the school, as well as the available facilities. The study commenced on the 27th of November 2006 after being advertised in the school's newsletter. The total number of students contacted by letters, which were distributed at the school assemblies was 590; 196 in year 7, 186 in year 8 and 208 in year 9. These letters included all the relevant papers for both the parents/ guardians (letter, screening and consent form; Appendix IV.2) and the participants (information sheet, consent form; Appendix IV.3). Out of the 590 students approached 54 returned the forms (9% response rate); four did not consent (two males, two females) and eleven were ineligible (eight males

and four females) as assessed by their parents screening form. Out of the students that were excluded one had a learning disability, one had a mood disorder, two had colour blindness, one had peanut allergy, two did not want to have the breakfast meals, two had anaemia and two had chronic diseases (familial hyperlipidemia, long QT syndrome)'. As such, 39 students were screened, out of whom two were further excluded (two females, obese), nine students dropped out (three males, six females) and 28 completed all three visits. The last student at the school was tested on the 29th of March 2007.

Due to the low response rate from Dunraven, new schools were contacted at the beginning of February 2007, again by examining the schools' league tables (OFSTED). Two schools, Raynes Park High School, and Chestnut Grove School agreed to participate in the study. Raynes Park High School was the only school were not all students in years 7, 8 and 9 were contacted; 120 letters were distributed randomly by the form tutors to students in years 7 (n=40), 8 (n=40), and 9 (n=40). At Chestnut Grove School all 450 students were invited to take part, by giving a presentation at the year assemblies, 7 (n=150), 8 (n=149), and 9 (n=151), and distributing the envelopes to the students. The fieldwork at these two new schools commenced on the 27th and 28th of February, respectively. At Raynes Park High School only six dates were available to test children due to school exams, and other prior arrangements. Out of the 17 children that responded (14% response rate), one did not consent (female), and 16 were screened; out of the latter three were excluded (two males were obese and one female was underweight), three dropped out (two males, one female), and one we did not have enough time to test (female). As such 9 children in total completed all three visits. Fieldwork at Raynes Park finished on the 21st March 2007. At Chestnut Grove School 35 students in total returned their forms (8% response rate); four did not consent (two males, two females) and two were ineligible (one male, one female, nut allergy) to take part as assessed by their parents' screening forms; 29 students were screened, out of whom 5 dropped out (two males, three females), 10 were excluded based on their screening appointment (three females (obese), seven males (one needed a translator, four were obese and two were underweight), and 14 completed all three visits. Fieldwork at Chestnut Grove was completed on the 10th May 2007.

Similarly, due to the low response rate from Raynes Park High School and Chestnut Grove, new schools had to be contacted by letter on the last week of April 2007. Two more schools, Battersea Technical College and Graveney School agreed to their participation in the study, following meetings with the schools' headteachers. Fieldwork commenced on the 17th May 2007 and 8th of June 2007, and finished on the 29th June 2007 and 6th July 2007, respectively. For both schools letters were distributed to all the students in years 7, 8 and 9, following a presentation at the school assemblies. The students at Battersea Technical college were 389 in total (131 in year 7, 128 in year 8, 130 in year 9), and at Graveney School 775 (259 in year 7, 262 in year 8, 254 in year 9). As far as the response rates are concerned, at Battersea Tech College 29 students returned their forms (7.5% response rate), out of whom seven were excluded based on their parents' screening form, and 22 were eligible to take part. Out of the 7 students that were excluded (two males, five females) one had a nut allergy, one never had breakfast, one had a learning disability, one had a mood disorder, one had cholelithiasis, one needed a translator, and one had Turner's syndrome). In total 21 students were screened, as one never showed up (male); five students were further excluded after their screening (all obese, one male, four females), three dropped out (females), and three were not tested due to limitations in time (females). Consequently, 10 students in total completed all three visits.

At Graveney School 68 students returned their forms (9% response rate), out of whom seven did not consent (six males, one female), and 13 were excluded based on their parents' screening forms. Out of the 13 students that were excluded (seven males, six females) four were allergic, one was obese, five had learning disabilities, one never had breakfast, and two had chronic diseases. Therefore, 48 students were eligible to participate in the study, out of whom 36 were screened due to limitations in time; five were excluded following their screening session (one male, four females, three were underweight, one was obese, and the other one had vision problems), and 12 were not feasible to be tested, again due to limitations in time (two males, ten females). Hence, 13 students in total completed all three visits. All children that were either excluded or there was not enough time to be tested, even if they had already agreed to their participation in the study, were informed by letter.

3.3.2.b Recruitment

As in the cross-sectional study, only students who returned the screening questionnaire and consent forms, signed by them and at least one parent or guardian, were eligible to take part. All information collected was held in strict confidence and all participants were given a unique identification number to ensure anonymity throughout the study. The screening questionnaire that the parents/ guardians of potential participants completed included the exclusion criteria, which were used to exclude students on medical or other grounds. The exclusion criteria are listed here:

- Sickle-cell anaemia, Haemophilia, Thalassaemia traits, or other blood disorders
- Colour blindness
- Diabetes or other glucose tolerance disorders
- Any other chronic diseases
- Learning disabilities or mood disorders
- Any medication that potentially affects glucose metabolism, mood, concentration, or cortisol levels
- Allergy or intolerance to any of the components of the breakfast meals (e.g. milk, nut, wheat)
- A parent/ guardian unwilling for their child to receive any of the preset meals
- Breakfast omitters (i.e. never have breakfast)
- Underweight or obese (BMI-for-age z-scores -2 SD or +2 SD, respectively) (WHO, 2007; Cole *et al.*, 2007)
- Non-native speakers (i.e. need translation); as that would affect their understanding, and effectively their performance on the CF tests
- Professional athletes or students taking part in exhaustive training

Therefore, a student was eligible to take part if the above selection criteria were met. Nonetheless, it is apparent that reported height and weight would pose a potential problem with regard to the calculation of BMI, and most importantly to the matching of the participants, which was based on both height and BMI (see section 3.3.3.b, page 101). It was thus decided, that all students that were preliminary eligible to take

part in the study, would be screened prior to the testing, in order to establish their height and weight.

3.3.3 Procedure

3.3.3.a Summary

Five schools were involved in the present study. In order for the study to be carried out effectively, certain facilities had to be provided on behalf of the schools. First of all, large enough rooms to screen and test children, as well as to store equipment. As such, in each school one main room was allocated from 08:00 until 11:30, where the equipment and food was stored. The breakfast administration either took place in this main room or in the school cafeteria (providing that it was unoccupied). Furthermore, one to two smaller rooms from 09:45 until 11:15, where the CF tests could be administered. This effectively means that two to three children could be tested each day. All the rooms were free from outside disturbances, wherever possible. As breakfast had to be prepared on the morning of the testing, food cupboards and space in the fridge was provided to store solid (corn flakes, muesli, sugar) and liquid food (milk, juice, water).

All participating students had to be seen three times: once for their screening (non-testing or 'normal' day), and twice for their testing day appointments ('breakfast' days). Appointment forms were prepared for both the screening day (Appendix IV.4) and the testing day (Appendix IV.5). These were left at the school, usually with the person that the headteacher had allocated to the study, to be given out to each child individually the day before their appointment. The appointment forms included instructions on what the students had to do the day before and on the morning of their appointment. The parents/ guardians of participating children were phoned as well the evening before their child's appointment, in order to confirm the appointment and to provide explanation on any of the instructions, if required. The non-testing and testing days are explained in detail in the following two sections.

3.3.3.b Non-testing day/ 'Normal' day

The non-testing day or 'normal' day as it was named for the sake of the participants, served screening purposes. Mainly to measure height and weight, and furthermore to take a saliva sample; the latter would be used to measure cortisol, a biomarker of stress. We wanted to take a baseline cortisol measure on a 'stress-free' day, so as to compare it to a 'stressful' day, when participants knew they would be tested. On a daily basis, children have to be at school by 08:30. Therefore, it would not be possible to see children any time before 08:00, as that would pose extra burden on the school, as well as on the children and their parents/ guardians. Besides, in young adults the meals were tested at approximately the same time, as their appointment was at 08:00. Thus, it was decided with the headteachers of the participating schools, that an appropriate time to make appointments with the children would be around 08:10. Researchers had to be at the school at least 15 minutes prior to the scheduled appointment to set everything up.

The day prior to their appointment children were asked to follow their normal routine and to have a good night's sleep (Appendix IV.4). On the morning of their appointment participants could have breakfast, if they wished, but they had to follow certain instructions, to avoid interference with cortisol levels (see section 3.3.3.g, page 107). Consumption of any food or drink that contained caffeine had to be avoided two hours prior to the appointment. Furthermore, 30 minutes before their appointments participants were instructed to avoid drinking or eating anything. Finally, children were instructed to avoid any form of strenuous exercise.

The non-testing day involved the following: First of all, the procedure was briefly explained to the child to ensure their understanding and consent to take part. A saliva sample was then taken, any time between 08:15 and 08:45 (30 minutes interval), usually between 08:15 and 08:30, but never before 08:15 or after 08:45. A brief interview followed, where questions were asked about how participants felt on that day, their breakfast on the morning of the study (if any), their eating habits, any vitamin supplements, any medication, their body image perception, the time they went to bed and the time they woke up, their physical activity levels, and their menstrual status (girls only). Finally, anthropometric measurements were taken. The Food Atlas

(Nelson *et al.*, 1997) was used to help children into quantifying the portion sizes of the foods/ drinks they had for breakfast. All measurements and responses were recorded in the researcher's booklet, non-testing day (Appendix IV.6).

3.3.3.c Matching of participants and randomization

Measured height and weight from the non-testing day was used to match children, and to calculate BMI, so as to exclude any children that were either underweight or obese. The matching criteria used were the following:

i. Same school year (7, 8 or 9)
ii. Same gender
iii. Height (± 3-5 cm)
iv. Age (± 6 months)
v. BMI (± 1 centile)
vi. Same school

All of these criteria had to be met, in order for a participant to be matched to another, with the exception of the same school. The matching criteria with regard to height and age, which are important markers of cognitive development, were based on the maximum growth velocity in adolescents, which is on average 9 cm per year (Tanner *et al.*, 1983). Therefore, 3-5 cm per 6 months was used as a matching criterion to approximate growth. Besides, CF tests are usually bracketed per 3 months (Wechsler, 1949). Wherever possible, participants from the same school were matched, as that would minimize the potential between-school differences. Nonetheless, due to the low response rate from all participating schools, this was not always feasible. When participants from different schools were matched, the matched pair was seen during the same school half-term.

Weight *per se* could not be used as a matching criterion; therefore, it was decided to use BMI as a marker to match children with similar height and weight. Children within the normal BMI range and ±1 centile apart are expected to have comparable height and weight, with normal physiological responses.

Each subject within a matched pair was randomly allocated to either the high or the low GL group, and received a pair number and a sequence number. Pair number was the same for each one of the two subjects within a matched pair, and pair numbers between 1 and 32 reflected all the matched subjects. The sequence number (1 to 64 for all the matched subjects) was indicative of the GL, and the order that the participants received the GI and the CF test version ('patterns'). There were four different 'patterns' for the two visits:

Patterns	1st visit		2nd visit	
	GI	CF version	GI	CF version
1	1	1	2	2
2	1	2	2	1
3	2	1	1	2
4	2	2	1	1

The order of the 'patterns' was randomized between all the pairs. Nonetheless, for subjects within the same pair the 'pattern' was the same. That is they received the GI and the CF version in the same order, to avoid any potential effects of GI or order of administration on the matched subjects.

3.3.3.d Testing day/ 'Breakfast day'

The wash-out period between the two testing days was two weeks. The aim was for the participants' testing days to be on the same day of the week, i.e. Monday and then again on a Monday two weeks later. This would minimize any effects of day-to-day variations on cognitive functioning and mood with regard to the normal routine. If a participant failed to come on his/ her scheduled visit, the appointment was rescheduled at his/ her earliest convenience. The testing days did not have to take place on the same day as the non-testing day. No subjects were tested the week before Christmas or Easter, due to possible effects of the anticipation of holidays on the subject's behaviour or to any changes in the school's routine (e.g. trips). The school's timetable was provided in order to be able to plan the testing days more effectively. Moreover, the same researcher tested the same child on his/ her two testing

appointments, wherever possible, in order to minimize the effect of between-researcher differences on the administration of the CF tests.

Participants had to follow certain instructions the day before and on the morning of their appointment (Appendix IV.5). The day before their appointments participants were asked to follow their normal routine, to avoid alcohol consumption, to restrict consumption of caffeine containing products, to restrict their participation in intense physical activity, and to have dinner which should be finished by 21:00. The latter would ensure that there was at least a 10 hour fast by the time they had their breakfast the following morning. The previous instructions were based on the recommendations for GI testing, so as to avoid any effects of the day before on the glycaemic response of the meal tested the next morning (Wolever *et al.*, 2003). For the same reason, participants were asked to record and consume the same dinner (or as similar as possible) the evening before their two testing appointments. Furthermore, children were asked to have a good night's sleep (~8 hours). On the morning of their testing day, participants were asked not to drink or eat anything (with the exception of water), and to avoid any form of strenuous physical activity.

The order of procedures was as follows:

x. Screening on the day, to ensure that the participant did not have anything to drink or eat
xi. Baseline saliva sample (between 08:15 and 08:45, usually between 08:15 and 08:30)
xii. Questions about how the participant was feeling on that day, and any physical activity prior to their appointment
xiii. Baseline finger prick blood sample, to measure BG and Hb
xiv. Baseline mood scales
xv. Breakfast administration (time zero)
xvi. Anthropometric measurements (Ht, Wt)
xvii. Interview

90 minutes after the start of breakfast

xviii. Saliva sample (before CF testing)
xix. Finger prick blood measurements of BG and Hb (before testing)
xx. Mood scales (before testing)

xxi. CF testing

xxii. Mood scales (after testing)

xxiii. Task demand questions

xxiv. Saliva sample (after testing)

xxv. Finger prick blood measurements of BG and Hb (after testing)

If screening on the day revealed that the student had something to eat or drink (with the exception of water), then he/ she was rescheduled at the earliest convenience. The 30 min maximum interval for the baseline saliva sample ensured that all subsequent saliva samples, as well as glucose measurements were taken with that 30 min interval for all participating students. Participants were not allowed to have anything to drink or eat (with the exception of water) during their testing day. As far as the interview questions asked were concerned, these were similar to the ones asked on the non-testing day: their dinner the evening before, any vitamin/ mineral supplements, any medication, if they were on a special diet for medical reasons, any infections, their physical activity on the day and on the previous day, the time they went to bed and woke up, and their menstrual status (girls only). This was done to ensure that if anything had changed between all three visits, it would be recorded and subsequently taken into account. The same Food Atlas (Nelson *et al.*, 1997) was used in the relevant questions.

All measurements and responses were recorded in the researcher's booklet, testing day (Appendix IV.7). At the end of each testing day, juice and water were provided to the children, a compliment slip addressed to their tutor to allow them to get back into class, and their next appointment form. On their next appointment form, the dinner they had the evening before their first visit was recorded by the researcher, in order for the participant to have the same dinner the evening before their next appointment. If it was the child's last visit a certificate of attendance was given to thank him/ her for their participation in the study (Appendix IV.8). Participating students were asked not to discuss the testing with any of their fellow students, until the testing/ study was completed in their school.

3.3.3.e Breakfast meals

Children were blinded to the meal they would receive. The meals were prepared on the morning of the study at each school by one of the researchers. Each individual food was weighed with food scales (CAS SW-1, Chasmores Ltd, UK) to the nearest 1g (weighing range of 0-2000g x 1g) to ensure that the amount (and therefore the GL and GI) was accurate to the original formulation. Children were instructed to consume the meal at a comfortable pace within 20 min, and to consume all food and drink provided. If the child could not finish the meal provided, he/ she was excluded from the study. Time zero was regarded as the time when eating commenced. It was then that a main timer was set, to indicate the start of the 90 min period between breakfast administration and CF testing. The time taken to finish the meal was accurately timed with a stopwatch and recorded. The foods that consisted the breakfast meals used, as well as the macronutrient analysis, GI and GL are presented in Table 3.3. The macronutrient composition of the breakfast meals is presented in Appendix IV.9. The meals were exactly the same as the ones used in the adult study, with the exception that in the high GL meals 7g of sugar were added instead of 10g, in order to obtain a better match for GI between meals for the intervention study. Such a small difference in the amount of sugar would not be expected to have an influence on any glucose or hormonal responses.

Table 3.3: Foods (g) that consisted the breakfast meals administered in the intervention study in children.

PRODUCTS (g)	BREAKFAST MEALS			
	HIGH GL		LOW GL	
	Low GI (M1)	High GI (M2b)	Low GI (M3)	High GI (M4)
GI meal	48	61	48	61
GL meal	41	55	21	28
Alpen Muesli no added sugar(Weetabix)	66	0	40	0
Kellogg's Corn Flakes	0	55	0	30
Tesco Semi-skimmed milk	200	300	250	300
Tesco Apple juice	245	200	0	0
Sugar white	7	7	5	5
Volume of liquid food	445	500	250	300
Water	55	0	250	200
Total volume	500			

3.3.3.f Anthropometric measurements

Height and weight were measured in all participating students, in all three visits. The same methodology and equipment were used as in the cross-sectional study (see section 3.1.4.b, page 72). Repeated measurements for the same subject were taken using the same set of scales on all three visits.

3.3.3.g Physiological measurements

❖ **Salivary Cortisol**

Salivary cortisol was measured at baseline on a 'stress-free' day, and on two 'stressful' days at three time-points: baseline, 90 and 150 min after the start of breakfast. The advantage of taking a sample on a 'stress-free' day, where the stressful stimuli, i.e. finger pricking, CF testing is not present, is that it can be used as a reference value. Due to the circadian and diurnal rhythms of cortisol, the time of day that the samples were collected had to be standardized. All samples were collected at a similar time of day for all participants, ideally 15 min apart. Wherever possible, the first sample was collected between 08:15 and 08:30. Nonetheless, in a research study involving children this is not always possible (e.g. delayed arrival, fire-alarms). This is why the time for the first sample was extended to include a 30 minute interval (between 08:15 and 08:45). Subsequent samples at 90 and 150 minutes after breakfast were taken with that 30 min maximum interval, as the entire procedure was carefully timed.

The sample collection device selected was the 'Salivette' with cotton swab without preparation (Starstedt Ltd), which is tasteless. 'Salivettes' were stored and used according to the manufacturer's specifications. Certain precautions were taken to ensure that cortisol levels would not be affected by the food eaten on that morning or by medication (Hanrahan *et al.*, 2006). The first questions asked on the non-testing day, as well as the questions with regard to any medication on all three visits served this purpose. To control for any possible effects of foods and/ or drinks on the cortisol levels, participants were asked not to eat or drink anything 30 min prior to their appointment, and to refrain from eating or drinking caffeinated products for two hours before their appointment (non-testing day). On the testing day participants should not have eaten or drank anything, so this was not an issue. Nonetheless, on all occasions

extra care was paid to ensure that no water had been drunk five minutes prior to sampling, as that might affect the natural oral environment, and salivary pH. Any subjects receiving oral steroids were excluded from the study (based on their parents' screening form), and the instructions were to exclude from the analysis any subject that had started taking oral steroids during the course of the study (this was never the case). Subjects were asked to keep the swabs in their mouth until they could no longer prevent themselves from swallowing the saliva produced (approximately 45 sec to 1 min). The time that the sample was placed in the mouth and then taken out of the mouth was recorded accordingly.

'Salivettes' were clearly labeled and frozen within 4-6 hours after their collection, and at -20^0C in batches. The samples were sent to King's College's Biochemistry Lab for analysis, once all the testing was completed, in order to minimize variation by using the same lot number of reagents. The biochemistry lab used a specifically adapted enzyme-linked immunosorbent assay (ELISA) to measure cortisol in saliva (Appendix IV.10).

❖ **Capillary Blood Glucose**

Capillary BG was measured using the AVIVA meter, which is a whole blood calibrated meter (see section 3.2.3.d.i, page 90). The protocol for repeated measurements has been described before (see section 3.1.4.c, Capillary BG, page 73). The two closest values were averaged. If the baseline BG reading was ≥5.6mmol/L, then that was an indication that the subject had something to eat or that he/ she had impaired glucose tolerance. Since the latter was unlikely, as all participants had normal weight, the participant was reinstructed to come back on another day. The importance of not eating or drinking anything on the morning of their testing day appointment was stressed. A lower end was also set, <3.9mmol/L, to avoid hypoglycaemia and any effects on brain function (i.e. neuroglycopenia).

❖ **Capillary Haemoglobin**

Whole blood Hb was measured using the 'HemoCue 201$^+$' (HemoCue Ltd, 2005) (see section 3.1.4.c, Capillary Haemoglobin, page 73). The same meter was used to take subsequent readings for the same participant. The two closest values were averaged.

3.3.3.h Mood scales

The same mood scales were used as in the cross-sectional study (see section 3.1.4.d, page 75). At each testing day, mood was assessed at baseline, before and after the CF tests.

3.3.3.i Cognitive function tests

The same battery of CF tests was administered as in the cross-sectional study (see section 3.1.4.e, page 76), and in the same order for the results to be comparable. Nonetheless, because each subject came back twice two versions were designed, CF 1 (similar as in cross-sectional study, Appendix II.7) and CF 2 (Appendix IV.11), the differences of which are presented in the table below:

CF TESTS	VERSION 1 (CF 1)	VERSION 2 (CF 2)
Word generation task	Letter T	Letter R
Word recall (immediate)	Different list of words	Different list of words
Stroop task	Same	Same
Matrices	Different set of patterns	Different set of patterns
Speed of information processing	Consecutive odd numbers	Consecutive even numbers
Serial sevens	Starting number 853	Starting number 635
Word recall (delayed)	Different list of words	Different list of words

For the word generation task, the selection of letters 'T' and 'R' was based on the fact that there is a comparable number of words beginning with either letter in the dictionary. With regard to the word recall both lists of words used in either version were of similar frequency and imagery (Appendix IV.12).

3.3.3.j Task demand questionnaire

Following the CF test battery and the mood scales ('after'), a task demand questionnaire was completed. This was the same as in the cross-sectional study (see section 3.1.4.f, page 79). Task demand was assessed on both testing days.

3.3.4 Data analysis

3.3.4.a Glycaemic Index and Glycaemic Load

The GI and GL of the dinner the evening prior to the testing day appointment was calculated as in section 3.1.5.a (page 81).

3.3.4.b Socio-economic classification and level of education

The screening form (Appendix IV.2) filled in by the parents provided information with regard to their socio-economic class and level of education; the latter was adapted from the Low Income Diet and Nutrition Survey (LIDNS) categorization (Nelson *et al.*, 2007). The National Statistics Socio-economic Classification (NS-SEC) of the parents/ guardians of each subject was assessed using the Standard Occupational classification (SOC) (Government Statistical Service, 2000). Parents/ guardians were asked to provide details on their employment status, i.e. whether an employer, self-employed or employee, whether a supervisor, and the number of employees at a workplace. This information was used to derive the occupation coded to unit groups (OUG) of SOC 2000. The latter was then used to allocate each parent/ guardian to one of the 17 operational categories of NS-SEC (14 functional and three residual), and to one of the eight analytic classes (AC). The eight AC are the following: (1) higher managerial and professional occupations, (2) lower managerial and professional occupations, (3) intermediate occupations, (4) small employers and own account workers, (5) lower supervisory and technical occupations, (6) semi-routine occupations, (7) routine occupations, (8) never worked and long-term unemployed. Long-term unemployment was defined as one year or over. The non-employed (i.e. retired, homemakers, sick, disabled) and full-time students were classified by their last main job. The NS-SEC was defined according to the highest of either the father or the mother.

3.3.4.c Nutrient analysis

The nutrient analysis of the foods consumed for breakfast on the non-testing day, and of the foods consumed for dinner the evening before a testing day, was calculated from the records of food consumption using a specially adapted Nutrient Databank, at the Department of Nutrition and Dietetics, King's College London. The Nutrient Databank was originally developed for the Ministry of Agriculture, Fisheries and Food (MAFF) for the NDNS of British adults (Gregory *et al.*, 1990). (The Nutrient Databank was transferred from MAFF to the Food Standards Agency (FSA) on its establishment in April 2000.) It was updated for the NDNS of children aged 1½-4½ years (Gregory *et al.*, 1995), people aged 65 years and over (Finch *et al.*, 1998), and young people aged 4-18 years (Gregory *et al.*, 2000). Further revisions and updates were carried out by FSA for the NDNS of adults aged 19-64 years (Henderson *et al.*, 2003). It was revised again by nutritionists at King's College London for the LIDNS (Nelson *et al.*, 2007) and the School Meals projects (Nelson *et al.*, 2004; Nelson *et al.*, 2006).

The databank contains nutritional information on over 8,000 foods and drinks, including manufactured products, homemade recipe dishes and many types of dietary supplements. Each food on the databank has values assigned for energy and 54 nutrients (Appendix IV.13). The nutrient values assigned to the foods in the databank are based on data from the Agency's rolling programme of nutrient analysis of foods. These data are also incorporated into the McCance and Widdowson's Composition of Foods series (FSA, 2002). A food code from the databank was allocated to each one of the foods consisting the meals eaten by all participants, which was then merged with the nutrient analysis per 100g for that food code from the databank. The nutrient analysis of the weight of food consumed by the pupils was then calculated.

3.3.4.d Physical activity

Physical activity was assessed as described in section 3.1.5.d (page 83). The information collected was the following: the participants' PA levels during a week (parent screening form), on the morning of their appointment and on the day before their appointment (non-testing, and testing). The information collected from the parents'/ guardians' screening forms was used to exclude students on the basis of excessive training.

3.3.5 Statistical analysis

Statistical analysis was conducted using the SPSS 15.0. The impact of GI and GL on blood glucose and salivary cortisol levels was assessed using repeated measures ANOVA; GI was a within-subject factor, and GL a between-subject factor. Further repeated measures analysis was carried out for each one of the mood states and the main CF test scores as dependent variables with main factors and covariates to determine the final prediction models. Details on each analytical model are given in the relevant sections in Chapter 4.

CHAPTER 4: RESULTS

4.1 Cross sectional study in school children

4.1.1 Grouping of the participants

The Power calculations required 30 participants in the low GI and 30 participants in the high GI group to acquire statistical significance. Therefore, participants were distributed into two GI groups; low and high. In order to compare the effects of the glycaemic potency of the different breakfast meals on CF, participants were further classified in four GI and GL groups according to whether their breakfast was above or below the median for GI=61 (60.64) and GL=27 (27.24) (Figure 4.1 and Figure 4.2, respectively). It was hypothesized that a low GI – high GL breakfast would be associated with the best CF, and that a high GI – low GL would be associated with the lowest CF; the other two breakfast types, low GI – low GL and high GI – high GL would be associated with intermediate levels of CF (i.e. High GL *vs.* Low GL, and Low GI *vs.* High GI associated with better performance). If a participant had a morning snack in addition to his/ her breakfast, the GI and GL of the snack was taken into account when calculating the overall GI and GL of the 'breakfast' consumed.

Figure 4.1: Distribution of the Glycaemic Index of breakfast.

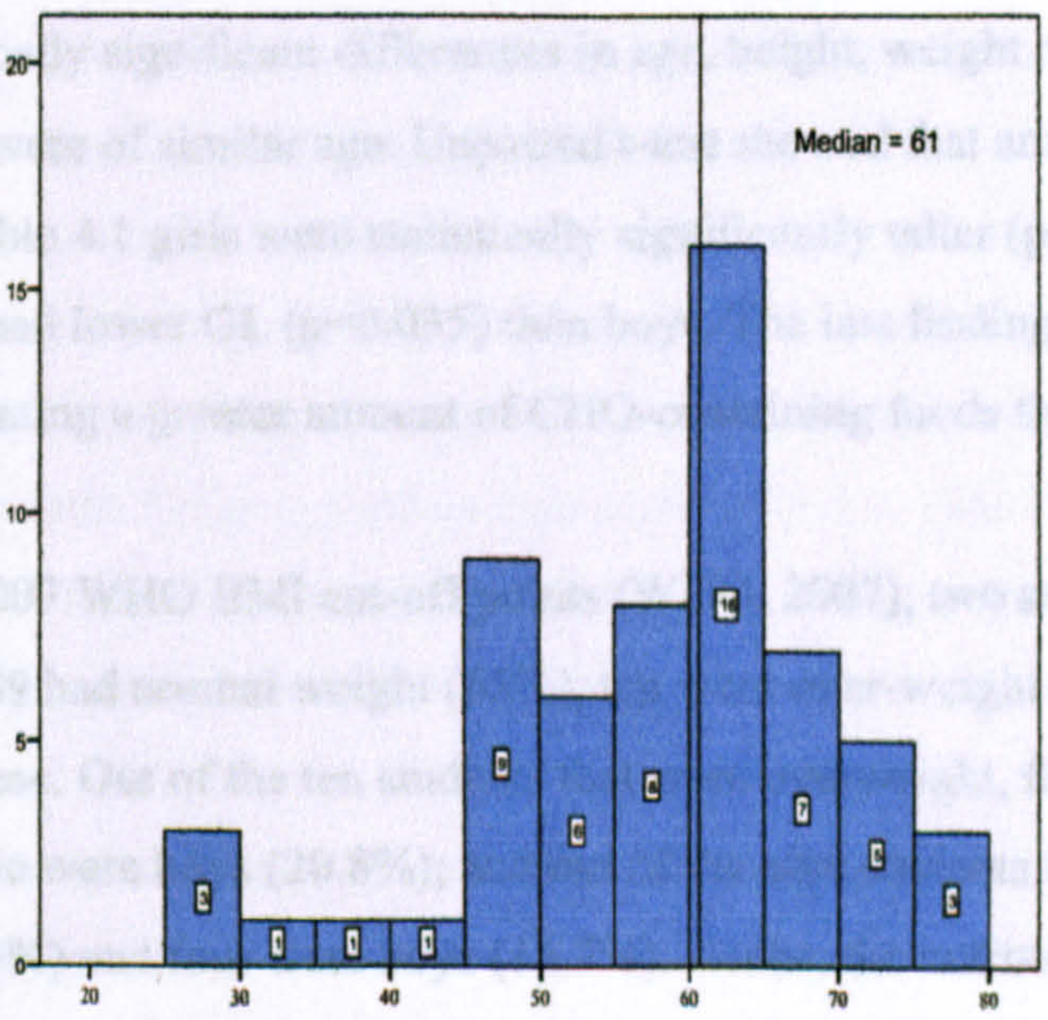
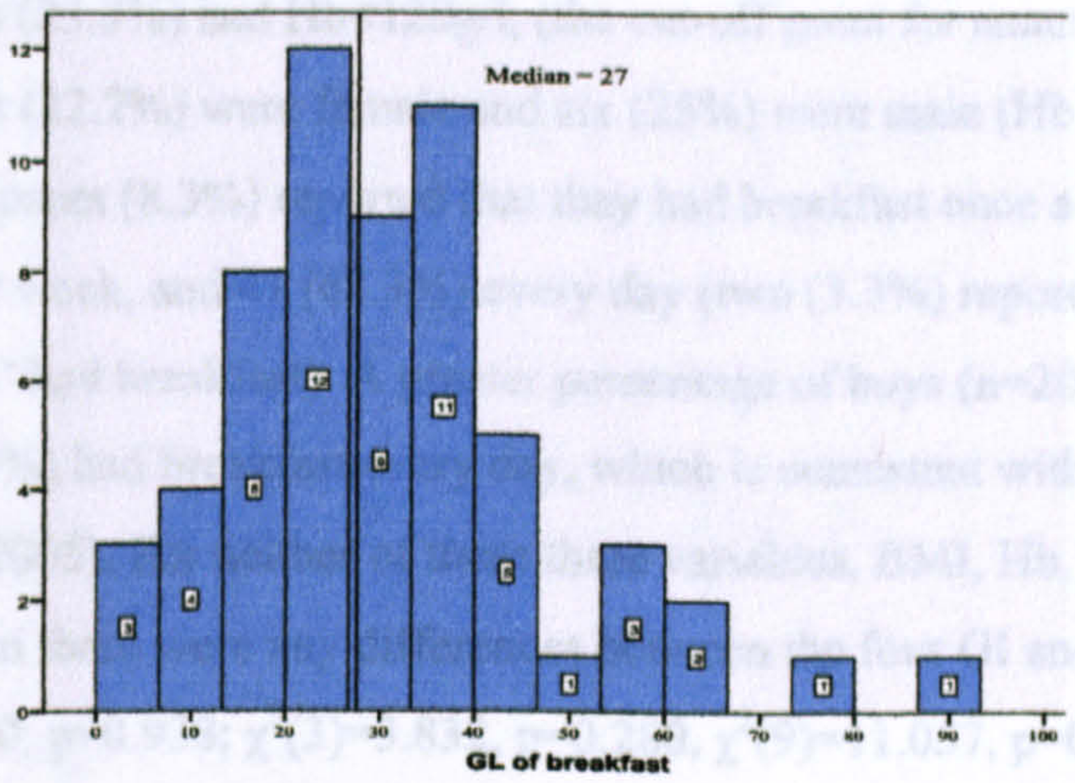


Figure 4.2: Distribution of the Glycaemic Load of breakfast.



4.1.2 Characteristics of the sample

Table 4.1 presents the descriptive characteristics of all the children, and of the children categorized in the four GI and GL groups. The meals correspond to either the 'breakfast' or the 'breakfast and snack' combined for the participants who had a substantial snack following breakfast. For simplicity, both meal types will be referred to as breakfast. In each of the low GI – high GL and the low GI – low GL groups, two children had a 'snack' as well; in the high GI – high GL, seven children had a 'snack', as did one child in the high GI – low GL. The four groups were well matched: there were no statistically significant differences in age, height, weight or BMI (Table 4.1). Boys and girls were of similar age. Unpaired t-test showed that among the variables presented in Table 4.1 girls were statistically significantly taller (p=0.012), heavier (p=0.042) and had lower GL (p=0.035) than boys. The last finding reflects simply that boys reported eating a greater amount of CHO-containing foods than girls.

Based on the 2007 WHO BMI cut-off points (WHO, 2007), two students (3.3%) were under-weight, 39 had normal weight (65%), ten were over-weight (16.7%) and nine (15%) were obese. Out of the ten students that were overweight, five were girls (13.9%) and five were boys (20.8%); and out of the nine students that were obese five were girls (13.9%) and four were boys (16.7%). Hence, the nutritional status of the sample, as characterized by the BMI z-scores, was similar for girls and boys ($\chi^2(3)=0.848$, p=0.838). None of the participants was dieting, and none of them was

stunted (height-for-age: <3rd centile). Twenty (55.5%) girls had started their periods; these observations were too few to be considered a potential confounder. Fourteen participants (23.3%) had Hb<120g/l, (the cut-off point for anaemia (WHO, 2001)), of whom eight (22.2%) were female and six (25%) were male (Hb range: 69-142g/l). Five participants (8.3%) reported that they had breakfast once a week, 12 (20%) twice or more per week, and 41 (68.3%) every day (two (3.3%) reported that they 'sometimes' had breakfast). A greater percentage of boys (n=20, 83.3%) than girls (n=21, 58.3%) had breakfast every day, which is consistent with another recent study (Sodexho, 2005). For neither of these three variables, BMI, Hb, and breakfast consumption there were any differences between the four GI and GL groups ($\chi^2(9)=3.560$, p=0.938; $\chi^2(3)=3.832$, p=0.280, $\chi^2(9)=11.057$, p=0.272, respectively).

Thirty one children (51.7%) were White, 16 (26.7%) were Black, and 13 (21.7%) from other ethnic backgrounds (mixed races). There were no statistically significant differences in the distribution of ethnic groups between the four GI and GL groups ($\chi^2(6)=10.231$, p=0.115). Within the four GI – GL groups, the relevant frequencies were as follows: in the low GI – high GL group seven children (63.6%) were white, one (9.1%) was Black, and three (27.3%) from other ethnic backgrounds; in the low GI – low GL group nine (47.4%), three (15.8%), and seven (36.8%); in the high GI – high GL group nine (47.4%), nine (47.4%), and one (5.3%); and in the high GI – low GL group six (54.5%), three (27.3%), and two (8.2%), respectively. Most parents/ guardians (n=22, 36.7%) were in SEG 10 (i.e. semi-skilled manual workers) or SEG 2 (n=9, 15%) (i.e. employers and managers in industry). In the low GI – high GL group, the majority of parents was in SEG 2 (n=4/ 11, 36.4%). For the other groups the largest proportion was SEG 10; low GI – low GL (n=8/ 19, 42.1%), high GI – high GL (n=7/ 19, 36.8%) and high GI – low GL (n=5/ 11, 45.5%). When the SEG was categorized into three larger groupings (see section 3.1.5.b, page 82) there were no statistically significant differences in the distribution of social classes between the four GI and GL groups ($\chi^2(6)=2.989$, p=0.810).

Overall, it appears that the four GI and GL groups as classified by the median for GI and GL were well matched; that is, there were no differences in the distribution of age, height, weight, BMI-for-age, Hb, usual breakfast consumption, ethnic group and SEG between these four groups.

Table 4.1: Descriptive characteristics, GI – GL values and finger prick blood measures in 60 children participating in the study, all children, and in the four GI and GL groups, by gender.

	Females		Males		All children		Low GI – High GL		High GI – High GL		Low GI – Low GL		High GI – Low GL		
n	36		24		60		11		19		19		11		
(females/males)					(36/24)		(3/8)		(12/7)		(13/6)		(8/3)		
	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	p‡
Age (y)	13.0	0.1	12.9	0.1	12.9	0.1	13.2	0.1	12.8	0.1	13	0.1	12.8	0.2	0.294
Height (cm)	159.5	1.1	154.2	1.8	157.4	1.0	157.9	2.9	157.6	1.9	154.6	1.7	161.1	1.6	0.199
Weight (kg)	53.0	2.0	46.8	2.1	50.6	1.5	49.9	2.6	50	2.8	49.2	2.8	54.6	3.9	0.651
BMI (kg/m^2)	20.7	0.6	19.6	0.7	20.3	0.5	20	0.8	19.9	0.8	20.4	0.9	21	1.4	0.895
GI*	57	2	58	3	57.6	2	53	1	68	1	46	2	64	1	***<0.001***
GL*	27	2	37	4	31.0	2	43	3	44	4	16	2	23	1	***<0.001***
Hb (g/l) †	125.9	2.1	128.2	2	126.8	1.49	127.1	6.5	126.6	1.6	128.3	1.9	124.2	3	0.828
BG before test battery (mg/dl) †	100.7	1.6	105.2	2.6	102.5	1.41	104.7	3.5	106.2	2.7	100.1	2.2	98.2	2.9	0.155
BG after test battery (mg/dl) †	100.9	2.0	107.5	3.2	103.5	1.79	100.8	2.8	104.3	3.6	106.0	3.0	100.7	4.8	0.686
Difference in BG (mg/dl) † (after test-before test)	0.2	2.1	2.3	3.2	1.0	1.79	-3.9	3.2	-1.9	3.8	5.9	2.9	2.5	3.8	0.193

* Glycaemic Index (GI) and Glycaemic Load (GL) values corresponding to breakfast or breakfast plus snack (reference food: glucose).

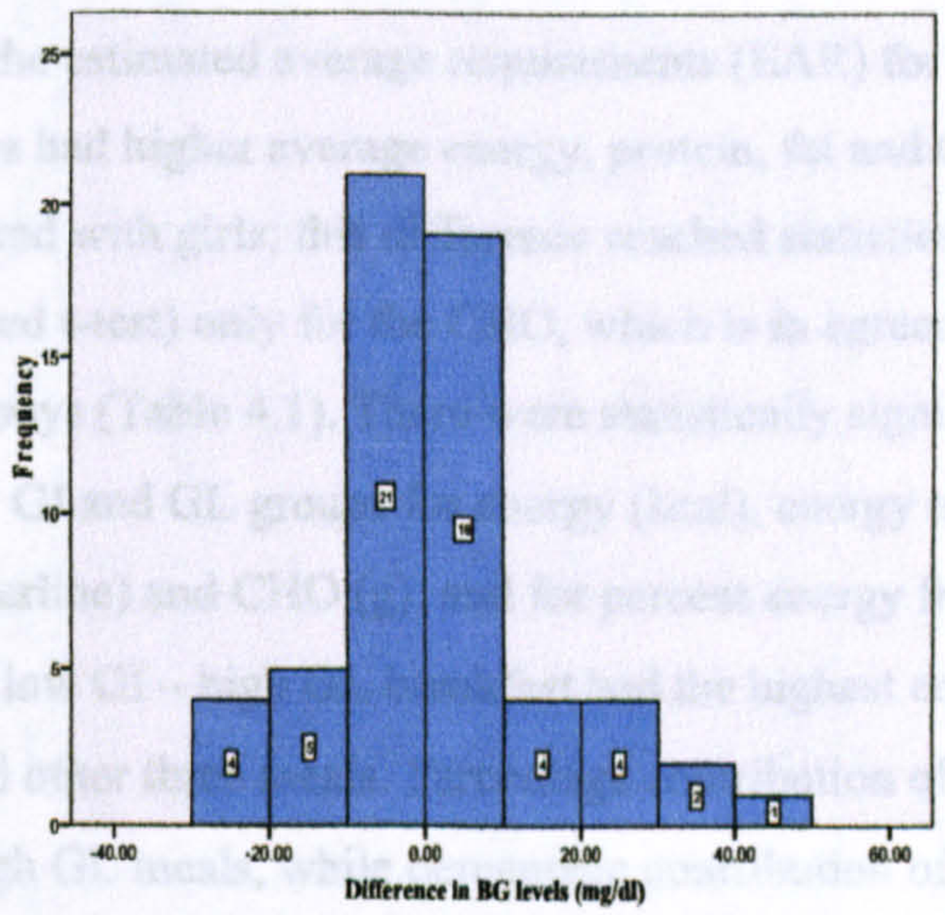
† Finger prick blood measurements of haemoglobin (Hb), and blood glucose (BG).

‡ One-way analysis of variance (ANOVA).

Two-tailed significance ***<0.05***.

Blood glucose levels before and after the tests were within ±20mg/dl for the majority of the pupils (n=49). Almost half of the children's BG levels increased after the tests, while for the remainder it fell (Figure 4.3); mean change in BG was 1.0mg (se=1.8). If on average half of the students' BG levels were rising and for the other half it was dropping, it suggests that the correct time-point (i.e. 90-120 min after breakfast) was identified to administer the CF tests.

Figure 4.3: Distribution of the difference in blood glucose levels, taken immediately before and immediately after the CF tests.



The mean GI and GL values were 57.6 (se=1.5) and 31.0 (se=2.2), respectively. There were no statistically significant differences in the mean values for any of the variables presented in Table 4.1 between the four GI and GL groups, apart from the GI and GL (which was of course expected). Mean BG in the high GL groups fell after the CF tests, while mean BG in the low GL groups rose, but the differences in the changes between the four GI – GL groups were not statistically significant. When participants were divided into two groups above or below the median for GL, however, BG was higher in the high GL group (105.6±2.1 *vs.* 99.4±1.7 mg/dl) before the tests (p=0.025, unpaired t-test), and the fall in BG in the high GL (-2.6±2.6 mg/dl) group was statistically significantly different from the rise in BG in the low GL group (4.7±2.3 mg/dl) (p=0.040, unpaired t-test). These two findings support the original hypothesis that between 90-120 min after breakfast and the completion of the CF tests there was

a rise in BG levels for the low GL group (suggesting a recovery from below the baseline), while for the high GL group there was a drop in BG levels (suggesting that BG was returning to baseline). When participants were divided into two groups above or below the median for GI, there were no differences in BG levels ('before', 'after', and 'after minus before') between the two GI groups (unpaired t-test).

4.1.3 Macronutrient composition of breakfast meals

The macronutrient composition of breakfast and of the breakfast meals corresponding to the GI and GL classification is presented in Table 4.2. Breakfast contributed on average 17% to the estimated average requirements (EAR) for both boys and girls (DH, 1991). Boys had higher average energy, protein, fat and CHO intakes at breakfast compared with girls; this difference reached statistical significance ($p=0.021$, unpaired t-test) only for the CHO, which is in agreement with the higher GL observed in boys (Table 4.1). There were statistically significant differences between the four GI and GL groups for energy (kcal), energy as percentage of EAR, protein (g) (borderline) and CHO (g), and for percent energy from fat and CHO (Table 4.2). The low GI – high GL breakfast had the highest energy content in comparison to all other three meals. Percentage contribution of CHO to energy was higher for the high GL meals, while percentage contribution of fat to energy was higher for the low GL meals. Table 4.3 shows examples of the breakfast meals corresponding to the GI and GL classifications, as reported by the participants.

Table 4.2: Macronutrient composition of breakfast* in 60 children participating in the study, all children, and in the four GI and GL groups, by gender.

	Females		Males		All children		Low GI, High GL		High GI, High GL		Low GI, Low GL		High GI, Low GL		
n (females/ males)	36		24		60 (36/24)		11 (3/8)		19 (12/7)		19 (13/6)		11 (8/3)		
	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	p‡
Energy (kcal)	307.8	25.15	393.6	42.68	342.2	23.22	502.2	63.2	378.7	39.83	272	32.75	240.3	20.26	*<0.001*
Energy (kj)	1287.8	105.23	1647	178.58	1431.6	97.15	2101.1	264.43	1584.3	166.64	1138	137.05	1005.4	84.78	
Breakfast contribution to EAR (%) †	16.7	1.36	17.7	1.92	17.1	1.11	24.2	3.42	18.9	1.69	13.9	1.66	12.5	1.19	*0.002*
Protein g	10.8	1.06	14.6	2.07	12.3	1.06	17.9	2.91	12.6	2.17	10.2	1.57	9.7	0.95	0.053
Protein (% energy)	13.9	0.81	14.5	0.69	14.2	0.56	13.8	1.1	13	0.95	14.4	1.2	16.1	0.93	0.302
Fat g	10	1.35	11.2	2.18	10.5	1.18	14	3.5	9.3	1.59	11.3	2.46	7.7	1.84	0.376
Fat (% energy)	27.9	2.37	24.1	2.71	26.3	1.79	22.1	2.99	20.8	1.67	34.5	4.1	26.2	3.85	*0.011*
Carbohydrate g	46.6	3.61	62.5	6.2	52.9	3.41	81.2	6.04	65.2	5.54	34.5	3.73	35.4	1.9	*<0.001*
Carbohydrate (% energy)	58.2	2.42	61.5	3.01	59.5	1.88	64.1	3.13	66.2	1.86	51.1	4.29	57.7	3.69	*0.006*

*The term 'breakfast' refers to both the 'breakfast' eaten by the children at their homes or to the combined 'breakfast' and 'snack', where appropriate.

† EAR: Estimated Average Requirements (DH, 1991).

‡ One-way analysis of variance (ANOVA).

Two-tailed significance *<0.05*.

Table 4.3: Examples of breakfast meals corresponding to the GI and GL classification.

Low GI – High GL	High GI – High GL	Low GI – Low GL	High GI – Low GL
Special K/ Muesli / Fruit and Fiber Semi-skimmed milk Orange juice Sugar	Corn Flakes/ Coco pops/ Rice Krispies/ Cheerios Semi-skimmed milk Sugar Orange juice	Special K/ Shredded Wheat Semi-skimmed milk Sugar	Cheerios/ Crunchy Nut Corn Flakes Semi-skimmed milk/ Chocolate milk
		Porridge Orange juice Whole meal bread Jam Butter	

4.1.4 Cognitive Function and possible predictors

It is important for the data to be explored before deciding which statistical test is appropriate. Therefore, if parametric tests are to be used then the assumption of normality needs to be tested. In order to test this assumption, frequency distributions, the Kolmogorov-Smirnov test (K-S) (normal distribution), and the Levene's test (homogeneity of variance; the spread of scores should be the same after each meal) were carried out for the main outcome variables, the CF test scores. All the tests were carried out separately for males and females. The histogram can reveal information about the distribution of the data, and hence it was the first test that was carried out. Just the main variables for each one of the seven tests were used to carry out the K-S test; word generation task 'total correct', immediate word recall 'total correct', time to complete the 'actual' Stroop task, matrices 'total correct', speed of information processing 'total correct', serial sevens 'total correct', and delayed word recall 'total correct'. The tests that were not normally distributed were the matrices, the speed of information processing, the serial sevens (just for females), and the delayed word recall. There were no successful transformations that normalised these test scores. In spite of the K-S test and the transformation of the original data suggesting that these were not normal, examination of the histograms revealed no strong kurtosis or skewness that would seriously undermine the use of parametric approaches to the analysis. Besides, using parametric tests is the only way to take the confounders into account. Furthermore, it would be extremely difficult to use different tests (parametric, non-parametric) for a battery of tests that are used to measure the same outcome, in our case the effect of the glycaemic potency of breakfast on CF. So, parametric tests were used for the CF test scores.

The predictors included the descriptive characteristics (age, height, weight, BMI), gender, the finger prick blood measurements (BG and Hb), the energy load and macronutrient composition of the breakfast, including the GI and GL, and other variables such as the GI and GL of the last meal the evening before, the hours of sleep, time between breakfast and the first CF test, time between waking up and the first CF test, exercise on the day and the day before. Correlation analysis was carried out to assess linear relationships between the CF test scores and possible predictors.

Average time between breakfast ingestion and the first CF test was 113.2 min (se=3.0), and between waking up and the first CF test was 149 min (se=3.9). Average time between breakfast ingestion and the first finger prick was 105.1 min (se=3.1), and the mean time difference between the first and the second finger prick was 44.1 min (se=3.3). The latter effectively represents the average time the whole testing procedure lasted with each child. The mean hours of sleep were 8.3 (se=0.2). The mean GI and GL of the dinner the night before was 52 (se=1.6) and 46 (se=3.9), respectively. Unpaired t-test revealed that there were no differences between males and females as far as these other variables are concerned, with the exception of PA the day before, where boys exercised more than girls (p=0.046, unpaired t-test).

Pearson correlation coefficients between the CF test scores and possible predictors are presented in Table 4.4. The key variables were used to identify potential predictors. The number of correlations performed were 8 (number of test scores variables) x 22 (number of potential predictors) = 176. This effectively means that nine correlations would be expected to come up by chance at the 5% significance level, one or two at the 1%, and perhaps 1 at the 0.1%. The number of significant correlations that came up is 20, which is about double the number of correlations someone would expect to observe by chance. It seems that the most important predictors were age, height, gender, and the GI and GL of the dinner the night before the testing.

Weight was unrelated to the CF test scores; similarly, the macronutrient composition of the breakfast meals, as well as the GI and GL of the breakfast meals and the hours of sleep. BMI was related to the 'interference' score of the Stroop task (i.e. time of the 'actual' test minus time of the 'control' test); the higher the BMI the higher the score, the longer it took the child to complete the 'actual' task compared with the 'control' task, that is worse inhibition. Older children performed on average better on serial sevens, and the Stroop task (quicker completion). Taller children (who were generally older) did better on the Stroop task, speed of information processing, and delayed recall. Males did better than females on the serial sevens task. Higher levels of Hb were associated with better performance on the word generation task (borderline significant p=0.05). The higher the BG levels after the CF test battery, the worse the performance on the Stroop task. Regarding the difference in BG, if BG was dropping (i.e. high GL groups), the bigger the drop the better the score on the Stroop task;

equally, if BG was rising (i.e. low GL groups), the bigger the rise the worse the performance on this test (borderline significant for the time to complete the 'actual' task p=0.05). Other significant predictors included the GI and GL of the dinner the night before, and the time since waking up and the first CF test. The higher the GI of the dinner the better the performance on the word generation, Stroop and delayed recall task. Similarly, the higher the GL of the dinner the night before, the better the pupils did on the word generation, Stroop and serial sevens task. Time since waking up was negatively associated with performance on the word generation task (borderline significant p=0.05), and the delayed recall task.

Table 4.4: Pearson Correlation Coefficients between CF test scores and possible predictors*.

	Age (y)	Height (cm)	BMI (kg/m^2)	Gender‡	Hb (g/l)†	BG 'after' (mg/dl)†	Difference in BG (mg/dl)†	GI dinner	GL dinner	Time since waking up and first CF test
Word generation task 'correct'	0.172	0.252	-0.079	0.194	*0.256*[a]	-0.041	-0.104	*0.259*[a]	*0.284*[a]	*-0.260*[a]
Immediate word recall 'correct'	0.175	0.247	-0.049	-0.020	0.162	-0.212	-0.170	0.198	0.124	-0.197
Stroop task (sec)	*-0.455*[c]	*-0.305*[a]	0.173	0.063	-0.049	*0.256*[a]	*0.252*[a]	*-0.337*[b]	*-0.353*[b]	0.027
'Interference' score Stroop	*-0.262*[a]	-0.133	*0.312*[a]	0.028	0.091	0.210	*0.259*[a]	-0.247	*-0.270*[a]	-0.023
Matrices 'correct'	0.196	0.228	-0.101	-0.139	0.121	-0.060	-0.095	0.093	0.194	0.008
Speed of information processing 'correct'	0.188	*0.295*[a]	-0.074	0.039	0.139	-0.099	-0.101	0.192	0.039	0.009
Serial sevens 'correct'	*0.301*[a]	0.187	0.014	*0.464*[c]	0.116	-0.001	-0.076	0.096	*0.311*[a]	-0.119
Delayed word recall 'correct'	0.200	*0.322*[a]	-0.012	-0.026	0.106	-0.209	-0.243	*0.300*[a]	0.114	*-0.272*[a]

*Not all potential predictors are presented; only the ones that were significantly related to the cognitive function (CF) test scores.

† Finger prick blood measurements of Haemoglobin (Hb), and blood glucose (BG).

‡ Grouping: gender 1 Female, 2 Male.

Pearson correlation coefficients, $p \leq 0.05^a$, $p < 0.01^b$, $p < 0.001^c$.

4.1.5 Cognitive Function, Mood and Task Demand

❖ **Cognitive Function test scores**

The relationships between the CF test scores, mood and task demand were complex. The Mood Scales 'before' (MS1) could be considered as a potential predictor of the performance on the CF tests and the outcome of the meals eaten at breakfast, while the Mood Scales 'after' (MS2) as an outcome of both the CF testing and the meals eaten at breakfast. As it is impossible to disentangle the combined effects of both the testing and of the meals on the MS2, MS1 will be considered as the predictor out of the two mood scales. Nonetheless, it would be worth seeing how the children reported feeling both before and after the tests, and how that was related to their performance. The number of correlations performed were 8 (number of test scores variables) x 22 (number of mood states either 'before' or 'after') = 176. This means, as before, that nine correlations would be expected to come up by chance at the 5% significance level, one or two at the 1%, and perhaps one at the 0.1%. The number of significant correlations that came up for the MS1 were nine, which implies that these could have well arisen by chance. For the MS2, the number of significant correlations at the 5% level was 16, which suggests that it is unlikely for all of these to have arisen by chance. In general, before the tests 'positive' feelings such as 'friendly', 'happy', 'relaxed' and 'calm' were associated with lower scores on a number of CF tests, while after the tests 'negative' feelings such as 'drowsy', 'tired' and 'sluggish' were associated with higher scores. Pearson correlation coefficients between CF scores and mood scales are presented in Table 4.5 (mood scales 'before' – MS1) and Table 4.6 (mood scales 'after' – MS2). Specifically, for each one of the tests performance was associated with the following mood states both before and after the CF tests:

	'BEFORE' (MS1)	'AFTER' (MS2)
Word generation task 'correct'	↓ Angry	↑ Drowsy ↑ Sluggish ↓ Lively
Immediate word recall 'correct'	↓ Happy	↓ Friendly ↓ Calm ↑ Drowsy ↑ Tired
Stroop task	↓ Sad ↓ Dissatisfied	↓ Sad ↓ Dissatisfied
Matrices 'correct'	↑ Nervous	↑ Sluggish
Speed of information processing 'correct'	↓ Friendly ↓ Happy ↓ Dissatisfied	↓ Uncertain ↓ Contented ↑ Thirsty
Serial sevens 'correct'	None	↓ Friendly ↓ Happy ↓ Muddled ↓ Dissatisfied
Delayed word recall 'correct'	↓ Happy ↓ Relaxed	

↑/↓: Better/ Worse performance at higher reported feelings of a particular mood state, respectively.

Table 4.5: Pearson Correlation Coefficients between CF scores and Mood Scales before – MS1*.

	MOOD SCALES 'BEFORE'						
	Friendly	**Nervous**	**Happy**	**Sad**	**Relaxed**	**Dissatisfied**	**Angry**
Word generation task 'correct'	-0.188	0.160	-0.205	0.071	-0.077	-0.123	***-0.253**[a]*
Immediate word recall 'correct'	-0.225	0.078	***-0.323**[a]*	0.066	-0.212	0.109	0.037
Stroop task (sec)	-0.028	0.111	-0.067	***0.275**[a]*	0.084	***0.286**[a]*	0.249
'Interference' score Stroop	-0.088	0.013	-0.218	0.249	0.041	0.171	0.091
Matrices 'correct'	-0.033	***0.257**[a]*	-0.124	0.011	-0.102	-0.133	0.073
Speed of information processing 'correct'	***-0.268**[a]*	-0.093	***-0.266**[a]*	-0.041	-0.193	***-0.290**[a]*	-0.188
Serial sevens 'correct'	-0.204	-0.216	-0.183	-0.070	-0.120	-0.202	-0.182
Delayed word recall 'correct'	-0.163	0.233	***-0.342**[b]*	0.031	***-0.298**[a]*	0.126	0.064

*Not all mood states are presented; only the ones that were significantly related to the cognitive function (CF) test scores.

Pearson correlation coefficients, $p \leq 0.05^{a}$, $p < 0.01^{b}$, $p < 0.001^{c}$.

Table 4.6: Pearson Correlation Coefficients between CF scores and Mood Scales 'after' – MS2*.

	MOOD SCALES 'AFTER'												
	Friendly	**Drowsy**	**Happy**	**Calm**	**Uncertain**	**Sad**	**Muddled**	**Dissatisfied**	**Tired**	**Contented**	**Lively**	**Sluggish**	**Thirsty**
Word generation task 'correct'	-0.184	***0.255**[a]*	-0.084	-0.055	-0.098	0.121	-0.001	0.033	0.165	-0.127	***-0.262**[a]*	***0.260**[a]*	0.101
Immediate word recall 'correct'	***-0.318**[a]*	***0.300**[a]*	-0.203	***-0.307**[a]*	-0.030	-0.042	0.003	0.106	***0.268**[a]*	-0.081	-0.162	0.082	-0.029
Stroop task (sec)	-0.029	0.101	0.089	-0.049	0.184	***0.256**[a]*	0.099	***0.251**[a]*	0.115	0.144	0.031	-0.081	-0.004
'Interference' score Stroop	-0.166	0.157	-0.023	-0.023	0.023	0.222	0.053	0.162	0.200	0.098	-0.068	-0.055	-0.011
Matrices 'correct'	0.037	0.096	0.067	0.001	-0.182	-0.022	-0.018	-0.047	0.022	-0.050	-0.125	***0.282**[a]*	0.117
Speed of information processing 'correct'	-0.240	-0.017	-0.052	-0.038	***-0.307**[a]*	-0.079	-0.142	-0.088	0.076	***-0.300**[a]*	-0.046	0.016	***0.291**[a]*
Serial sevens 'correct'	***-0.325**[a]*	-0.008	***-0.265**[a]*	0.024	-0.228	-0.065	***-0.310**[a]*	***-0.258**[a]*	0.133	-0.227	-0.025	0.073	-0.011
Delayed word recall 'correct'	-0.189	0.131	-0.027	-0.241	-0.080	-0.092	-0.082	0.032	0.190	-0.048	-0.065	0.006	0.071

*Not all mood states are presented; only the ones that were significantly related to the cognitive function (CF) test scores.

Pearson correlation coefficients, $p \leq 0.05^{a}$, $p < 0.01^{b}$, $p < 0.001^{c}$.

❖ Mood

The relationships between mood and possible predictors were assessed by carrying out correlation analysis (Table 4.7 for MS1 and Table 4.8 for MS2). The number of correlations performed were 22 (number of mood states either 'before' or 'after') x 22 (number of potential predictors) = 484. This means, that 24 correlations would be expected to come up by chance at the 5% significance level, four or five at the 1%, and perhaps one at the 0.1%. The number of significant correlations that came up for MS1 was 30, which is more than the number of those that could have arisen by chance. At the 1% level there were eight significant correlations with age, gender, BG 'before', protein, CHO, the GL of the meal, time since breakfast and the first CF test, time since waking up and the first CF test, and PA on the morning of the testing; gender and time since waking up and the first CF test were significantly correlated with MS1 even at the 0.1% level. For MS2, the number of significant correlations at the 5% level was 30, which suggests that it is unlikely for all of these to have arisen by chance; four correlations with Hb, energy, protein, and the GL of the breakfast were significant at the 0.1% level. Nonetheless, it has to be kept in mind that the mood scales 'after' represent the effect of both the meals eaten at breakfast and of the CF testing on mood. The significant correlations are presented in the next three paragraphs. All the mood states **in bold** are correlated with potential predictors at the 1% level.

Overall, the relationships with certain predictors were inconsistent, not allowing for firm assumptions to be drawn. These predictors included gender, age, height (unrelated to either MS1 or MS2), weight, BMI, the hours of sleep, time between breakfast and the first CF test (min), time since waking up and the first CF test (min), PA on the day and the day before, as well as the GI and GL of the dinner the evening before.

Higher Hb levels were positively correlated with feelings of calmness before the tests, and negatively correlated with feeling 'friendly' before the tests, and **'uncertain' after** the tests. Blood glucose levels before the tests were not associated with MS1 or MS2, apart from feeling more **'nervous' 'before'** when BG was higher. Blood glucose levels after the tests were positively associated with nervousness, uncertainty,

and sadness. The consistent finding is that higher BG levels both before and after the tests were associated with feeling more 'nervous'.

As far as the macronutrient composition of the breakfast is concerned, higher energy (kcal), protein (g) and CHO (g) intakes at breakfast were associated with feeling more **'tense' before** the tests, and more 'nervous' and **'tense' after** the tests. In addition, higher energy intake was associated with feeling less 'friendly' and 'relaxed', and more 'sad' after the tests. Higher protein intake was also associated with feeling less 'friendly' after the tests. Higher CHO intake was in addition related to feeling less 'friendly', 'calm', and 'contented' before the tests; and less 'relaxed' after the tests. Higher fat (g) intake was associated with feeling more 'tense' after the tests. Glycaemic index was positively correlated with feeling 'energetic' before the tests. Glycaemic load was negatively correlated with feeling 'calm', 'contented' (as CHO (g)), and positively correlated with feeling 'tense' and 'uncertain' before the tests. **After** the tests, GL, was positively correlated with feeling 'nervous' and **'tense'** (as CHO (g)), and with feeling 'sad' and 'muddled'. Therefore, higher macronutrient intake and carbohydrate load at breakfast was associated with a state of nervousness and tenseness.

Finally, when one-way ANOVA was carried out to compare the means among the four groups of subjects with regard to MS1, pupils in the low GI – high GL group felt the least 'friendly' ($F(3,59)=7.765$, $p<0.001$), 'calm' (borderline) ($F(3,59)=0.071$, $p=0.071$), and 'energetic' ($F(3,59)=3.385$, $p=0.024$) before the CF tests (Table 4.9).

Table 4.7: Pearson Correlation Coefficients between Mood Scales (MS1) and possible predictors*.

	Age (y)	BMI (kg/m2)	Gender‡	Hb (g/l)	BG 'before' (mg/dl)†	Energy (kcal)	PRO (g)	FAT (g)	CHO (g)	GI of meal	GL of meal	GI dinner	Sleep (h)	Time between breakfast and first CF test (min)	Time since waking up and first CF test	PA today
Friendly	-0.047	-0.070	***-0.424****	***-0.327****	-0.029	-0.214	-0.186	-0.044	***-0.291****	0.085	-0.204	-0.035	***-0.279****	0.159	0.173	-0.146
Nervous	-0.138	-0.127	-0.007	0.023	***0.386****	0.011	0.012	-0.095	0.094	0.058	0.090	-0.051	-0.051	-0.053	-0.117	-0.100
Calm	-0.091	0.021	-0.145	***0.281****	-0.143	-0.159	-0.090	0.037	***-0.290****	-0.205	***-0.305****	-0.038	***0.310****	-0.170	-0.187	-0.020
Uncertain	-0.104	-0.118	0.249	0.006	0.215	0.162	0.190	0.012	0.221	0.124	***0.268****	-0.170	-0.122	-0.208	-0.209	-0.008
Sad	-0.146	-0.142	0.101	0.114	-0.097	0.168	0.183	0.173	0.100	0.010	0.107	-0.015	0.123	***-0.302****	-0.119	-0.104
Energetic	-0.174	-0.066	0.085	-0.165	0.096	0.077	0.035	0.148	0.004	***0.296****	0.104	0.149	0.211	0.042	0.064	-0.151
Muddled	-0.242	-0.021	-0.087	-0.076	-0.046	-0.079	0.040	-0.197	0.006	0.225	0.076	0.104	-0.080	***-0.255****	0.042	-0.152
Relaxed	-0.157	-0.055	-0.094	-0.059	0.177	0.041	0.080	0.120	-0.052	-0.001	-0.034	-0.021	0.034	0.028	0.184	***-0.349****
Confident	-0.217	0.109	-0.166	-0.201	0.105	0.037	0.108	0.099	-0.051	0.063	-0.015	0.103	-0.044	0.160	***0.417****	***-0.279****
Tired	***0.278****	0.035	0.178	0.036	0.187	-0.060	0.006	-0.167	0.028	-0.248	-0.080	0.057	-0.014	-0.148	-0.151	0.118
Angry	-0.145	-0.137	-0.103	0.040	-0.127	0.051	0.125	0.016	0.035	-0.188	-0.029	-0.074	-0.051	***-0.336****	0.010	-0.126
Contented	-0.094	0.081	-0.189	-0.213	-0.067	-0.202	-0.188	0.062	***-0.357****	-0.064	***-0.347****	***0.283****	0.095	0.202	0.152	-0.070
Lively	***-0.332****	-0.013	-0.061	-0.173	0.233	0.038	0.003	0.146	-0.054	0.169	0.003	0.153	0.106	0.128	0.177	-0.181
Tense	-0.153	-0.127	0.063	0.002	0.111	***0.326****	***0.374****	0.138	***0.323****	0.090	***0.307****	-0.120	-0.109	***-0.285****	-0.050	-0.185
Sluggish	-0.105	-0.116	***0.315****	0.027	0.201	-0.040	0.027	-0.155	0.048	-0.023	0.036	-0.062	-0.074	-0.203	-0.159	0.111
Clearheaded	0.041	-0.095	***-0.377****	-0.023	-0.009	-0.013	-0.088	0.144	-0.114	-0.124	-0.129	0.006	-0.038	0.143	0.186	-0.222
Hungry	-0.206	***-0.259****	-0.087	-0.120	0.062	-0.053	0.002	-0.036	-0.067	0.133	-0.028	-0.089	0.058	0.067	0.041	0.004

*Not all potential predictors and mood states are presented; only the ones that were significantly related to MS1.

† Finger prick blood measurements of Haemoglobin (Hb), and blood glucose (BG); ‡ Grouping: gender 1 Female, 2 Male.

Pearson correlation coefficients, $p \leq 0.05^a$, $p < 0.01^b$, $p < 0.001^c$.

Table 4.8: Pearson Correlation Coefficients between Mood Scales (MS2) and possible predictors*.

	Age (y)	Weight (kg)	BMI (kg/m2)	Hb (g/l)	BG 'after' (mg/dl)†	BG difference (mg/dl)	Energy (kcal)	PRO (g)	Fat (g)	CHO (g)	GL of meal	GL dinner	Sleep (h)	Time between breakfast and first CF test (min)	Time since waking up and first CF test (min)	PA today
Friendly	-0.125	-0.072	0.006	-0.119	0.199	0.134	*-0.274[a]*	*-0.312[a]*	-0.187	-0.238	-0.143	-0.131	*-0.281[a]*	0.101	0.132	-0.059
Nervous	-0.223	*-0.290[a]*	-0.240	-0.215	*0.278[a]*	0.090	*0.314[a]*	*0.293[a]*	0.230	*0.282[a]*	*0.324[a]*	-0.223	-0.026	0.215	0.190	-0.166
Uncertain	*-0.306[a]*	-0.250	-0.233	*-0.357[b]*	*0.265[a]*	0.119	0.161	0.156	0.114	0.145	0.228	-0.229	-0.051	0.124	0.076	-0.188
Sad	-0.186	-0.160	-0.110	0.058	*0.299[a]*	*0.305[a]*	*0.262[a]*	0.206	0.191	0.249	*0.306[a]*	-0.019	0.115	-0.148	-0.020	-0.034
Muddled	-0.151	-0.239	-0.247	-0.187	0.211	0.140	0.217	0.192	0.117	0.233	*0.324[a]*	-0.144	0.007	-0.081	-0.032	-0.029
Relaxed	0.053	0.205	0.165	0.035	-0.059	-0.032	*-0.271[a]*	-0.242	-0.158	*-0.281[a]*	-0.223	0.153	-0.026	-0.050	0.075	-0.171
Confident	-0.211	-0.088	-0.074	-0.037	0.179	0.034	-0.016	-0.024	0.043	-0.057	-0.022	-0.211	-0.046	0.066	*0.258[a]*	-0.071
Angry	-0.116	-0.162	-0.168	0.086	-0.076	0.055	0.107	0.180	0.069	0.077	0.039	-0.124	-0.034	*-0.275[a]*	0.059	-0.142
Tense	-0.219	-0.249	-0.223	-0.017	0.206	0.123	*0.381[b]*	*0.405[b]*	*0.289[a]*	*0.318[a]*	*0.392[b]*	-0.109	-0.004	-0.149	-0.069	*-0.280[a]*
Hungry	-0.130	*-0.300[a]*	*-0.327[a]*	-0.026	0.074	-0.008	0.037	-0.002	0.052	0.025	0.071	*-0.256[a]*	0.058	-0.045	-0.117	-0.010

*Not all potential predictors and mood states are presented; only the ones that were significantly related to MS2.

† Finger prick blood measurements of Haemoglobin (Hb), and blood glucose (BG).

Pearson correlation coefficients, $p \leq 0.05^a$, $p < 0.01^b$, $p < 0.001^c$.

Table 4.9: Comparison of Mood Scales 'before' (MS1) among the four GI and GL groups.

MS 1	HIGH GL				LOW GL				
	LOW GI		HIGH GI		LOW GI		HIGH GI		
n	11		19		19		11		
	Mean	se	Mean	se	Mean	Se	Mean	se	p*
Friendly	1.82	0.23	2.68	0.13	2.84	0.16	3.09	0.21	*<0.001*
Nervous	1.36	0.34	1.37	0.24	1.16	0.26	0.91	0.25	0.662
Drowsy	0.91	0.21	0.53	0.23	0.68	0.19	0.82	0.30	0.685
Happy	2.27	0.30	2.63	0.23	2.32	0.15	2.91	0.21	0.222
Calm	2.18	0.33	2.32	0.20	2.89	0.19	2.91	0.28	0.071
Uncertain	0.82	0.33	1.26	0.29	0.89	0.23	0.73	0.24	0.524
Sad	0.09	0.09	0.26	0.17	0.26	0.17	0.18	0.12	0.875
Energetic	1.55	0.37	2.58	0.25	1.95	0.24	2.73	0.30	*0.024*
Muddled	0.18	0.12	0.63	0.21	0.21	0.16	0.64	0.28	0.209
Relaxed	1.82	0.23	2.47	0.19	2.42	0.26	2.64	0.39	0.246
Dissatisfied	0.00	0.00	0.53	0.21	0.42	0.22	0.55	0.39	0.450
Alert	2.27	0.19	2.26	0.25	2.16	0.24	2.09	0.41	0.968
Confident	2.18	0.30	2.84	0.22	2.42	0.22	3.00	0.30	0.139
Tired	1.73	0.41	0.79	0.20	1.32	0.24	1.09	0.31	0.127
Angry	0.00	0.00	0.11	0.07	0.32	0.22	0.36	0.20	0.440
Contented	1.91	0.34	1.95	0.24	2.47	0.19	2.36	0.28	0.267
Lively	1.91	0.34	2.63	0.23	2.16	0.27	2.55	0.37	0.298
Tense	0.80	0.29	0.84	0.23	0.58	0.26	0.45	0.16	0.686
Sluggish	0.91	0.39	0.53	0.21	0.63	0.17	0.90	0.41	0.686
Clearheaded	1.82	0.30	2.11	0.30	2.11	0.31	1.55	0.37	0.616
Hungry	0.45	0.28	0.84	0.28	0.74	0.27	0.91	0.44	0.811
Thirsty	1.55	0.28	1.32	0.27	1.32	0.25	1.27	0.38	0.936

* One-way analysis of variance; Two-tailed significance, $p<0.05$, $0.05\leq p<0.08$.

❖ Task demand

Correlation analysis was carried out to reveal the relationships between self-reported task demand, effort, tiredness and CF. The number of correlations performed were 8 (number of test scores' variables) x 3 (demand, effort, tiredness) = 24. This means that one to two correlations would be expected to come up by chance at the 5% significance level, perhaps one at the 1%, and none at the 0.1%. The number of significant correlations that came up at the 5% level were seven, four at the 1%, and three at the 0.1% (all related with difficulty), which is unlikely to have arisen by chance. The self-reported task demand questionnaire can not be considered as a predictor of the performance on the CF test scores, as it was recorded on completion of the testing. It is merely a measure of the perceived cognitive demand, and can reveal associations between the former and performance.

In general, the more difficult these tests were perceived the lower the scores on the CF tests. This effect was statistically significant for all the tests, with the exception of the Stroop task and the matrices. Furthermore, the more effort the children put into recalling the word list (delayed) the better they did on this test ($r=0.303$, $p=0.019$). For all other tests (excluding the immediate word recall) there were observed negative associations between effort and performance, which were nonetheless non-significant. Finally, the more tiring the children perceived the tests to be the less well they performed; this negative correlation reached statistical significance for the delayed word recall ($r=-0.353$, $p=0.006$).

Correlation analysis between task demand questions and possible predictors revealed a few significant values. Specifically, the number of correlations performed was 24 (demand, effort, tiredness for all eight CF test variables) x 22 (number of potential predictors) = 528. This means that 26 correlations would be expected to come up by chance at the 5% significance level, five at the 1%, and perhaps one at the 0.1%. The number of significant correlations that came up at the 5% level was 16, three at the 1%, and none at the 0.1%. As there were so few significant correlations from so many possibilities, the correlations seen here are probably due to chance. This suggests that the physiological parameters and other variables had no significant effect on how difficult and tiring the child perceived each test to be, or how much effort he/ she put into doing it.

4.1.6 Cognitive Function and breakfast meals

One-way ANOVA was performed to compare the means among the four groups of participants with regard to their performance on the CF tests. The low GI – high GL group performed better on all of the CF tests except for memory, immediate and delayed; but only for two of these, speed of information processing ($F(3,56)=3.685$, $p<0.05$), and serial sevens ($F(3,56)=4.370$, $p<0.001$), the differences were statistically significant (Table 4.10: all possible variables are presented, not just the main CF test variables). There were no significant differences between boys and girls in their performance on the CF tests, apart from the serial sevens task in which boys performed better than girls ($p=0.001$, unpaired t-test). Social class and usual breakfast consumption were unrelated to performance on the CF tests.

Based on pupils' ratings (0-4), serial sevens was the most difficult task (2.97, se=0.12), followed by delayed recall (2.65, se=0.14), speed of information processing (2.18, se=0.13), immediate recall (2.13, se=0.11), word generation (2.02, se=0.10), Stroop task (1.95, se=0.12), and matrices (1.60, se=0.13) (Friedman test, $p<0.001$).

Table 4.10: Comparison of cognitive function test scores* among the four GI and GL groups.

	HIGH GL				LOW GL				
	LOW GI		HIGH GI		LOW GI		HIGH GI		
n	11		19		19		11		
	Mean	se	Mean	se	Mean	se	Mean	se	p†
Word generation task 'correct'	20.2	1.9	18.4	1.3	16.8	0.9	16.9	1.7	0.359
• 'errors'	0.2	0.1	0.5	0.2	0.5	0.1	0.4	0.2	0.705
• 'correct-errors'	20.0	1.9	17.9	1.3	16.4	1.0	16.5	1.7	0.302
Word recall immediate 'correct'	7.5	0.9	8.2	0.6	7.6	0.5	8.6	0.5	0.612
• 'errors'	0.6	0.4	0.3	0.2	0.2	0.1	0.3	0.1	0.508
• 'correct-errors'	6.8	1.0	7.8	0.7	7.4	0.6	8.4	0.5	0.574
Stroop task 'control' time	71.3	2.9	80.6	3.5	80.4	2.9	76.8	4.9	0.275
• 'actual' time	42.8	1.8	47.4	1.9	45.4	1.6	45.5	3.3	0.533
• 'interference' score‡	28.5	2.0	33.2	3.3	35.1	2.6	31.3	3.5	0.496
• 'correct'	59.1	0.5	58.3	0.6	58.7	0.3	59.1	0.4	0.670
• 'errors'	0.9	0.5	1.7	0.6	1.3	0.3	0.9	0.4	0.670
• 'correct-errors'	58.2	1.1	56.6	1.3	57.5	0.6	58.2	0.7	0.670
Matrices 'correct'	13.2	0.4	12.6	0.4	12.5	0.4	11.5	0.7	0.227
Speed of information processing 'correct'	13.9	1.1	13.0	0.7	12.1	0.6	9.8	0.9	***0.017***
• 'accuracy' score	0.9	0.0	0.9	0.0	0.9	0.0	0.7	0.1	0.108
• 'misses'	1.9	0.9	3.4	2.1	1.6	0.5	9.4	6.7	0.267
• 'errors'	0.3	0.1	0.5	0.4	0.5	0.3	0.3	0.1	0.920
• 'correct-errors'	13.6	1.2	12.5	0.8	11.6	0.8	9.5	0.9	***0.047***
Serial sevens 'correct'	29.3	5.3	15.1	2.1	15.6	2.2	18.0	2.7	***0.008***
• 'errors'	4.6	1.5	5.2	1.3	3.7	0.6	4.6	1.0	0.765
• 'correct-errors'	24.6	6.4	9.9	2.8	11.8	2.4	13.4	3.4	***0.042***
Word recall delayed 'correct'	6.5	1.2	6.2	0.7	5.8	0.6	6.6	0.5	0.895
• 'errors'	0.4	0.4	0.3	0.1	0.4	0.1	0.5	0.3	0.933
• 'correct-errors'	6.1	1.3	5.9	0.7	5.5	0.7	6.2	0.7	0.927
• 'forgetting'**	1.0	0.6	1.9	0.4	1.7	0.4	2.0	0.5	0.550

* All possible CF test scores' variables are presented; † One-way analysis of variance; ‡ Stroop task 'actual' minus 'control' time; **Word recall immediate 'correct' minus word recall delayed 'correct'; Two-tailed significance, $p<0.05$.

Further ANOVA was carried out for each one of the CF test scores (main variables) as dependent variables taking three factors into account: GI and GL (below and above the median); and gender. In order to identify covariates for inclusion in the ANOVA, correlation analysis was carried out to determine linear associations between the CF test scores and possible predictors (see section 4.1.4, page 121). The significant predictors from the correlation analysis (age, height, weight, BMI, Hb levels, BG levels, time between waking up and the first CF test and feeling 'happy' before the CF tests; the GI and the GL of the meal the evening before were not entered as it is unlikely to have predicted performance on the CF tests) were entered in stepwise multiple regression analysis for each one of the CF tests. Any of the variables that were significantly associated with the CF tests in the multiple regression analysis were included initially as covariates in all of the ANOVA models. The ANOVA model for each CF test was then further refined, removing the non-significant interactions first (starting with the non-significant interaction with the highest p-value), then removing the non-significant factors and covariates, until all of the non-significant interactions, factors and covariates had been removed from the model. The non-main CF test variables were unrelated to GI, GL or GI and GL. The findings below give results for the ANOVA taking all main factors and relevant covariates into account.

Word generation task

GI, GL and gender did not appear in the model as significant factors; multiple regression analysis revealed that the length of the interval between waking up and the first CF test was negatively associated with performance ($F(1,58)=4.203$, $p=0.041$ $\Delta R^2=5.1$).

Immediate recall

Performance on this test was predicted by both GI, and feeling 'happy' before the tests ($F(1,57)=4.371$, $p=0.045$, and $F(1,57)=9.665$, $p=0.003$, respectively; final model: $F(2,57)=5.752$, $p=0.005$, $\Delta R^2=13.9$); the lower the GI of the breakfast and the happier the child felt before the CF tests, the worse the performance on this test.

Stroop task

Older children took less time to complete the Stroop task, and BMI was negatively associated with Stroop performance ($F(1,57)=17.716$, $p<0.001$, and $F(1,57)=4.045$, $p=0.049$, respectively; final model: $F(2,57)=10.013$, $p=0.001$, $\Delta R^2=23.4$). Similarly, age and BMI were associated with the 'interference' score; that is older children, and children who had lower BMI performed better ($F(1,57)=6.365$, $p=0.014$, and $F(1,57)=8.383$, $p=0.005$, respectively; final model: $F(2,57)=6.596$, $p=0.003$, $\Delta R^2=15.9$). Finally, for the 'control' task older children performed better ($F(1,58)=11.254$, $p=0.001$, $\Delta R^2=14.8$).

Matrices

Matrices performance was associated with GL, gender, and their interaction ($F(1,56)=6.665$, $p=0.012$, borderline: $F(1,56)=3.360$, $p=0.072$, and $F(1,56)=11.793$, $p=0.001$, respectively; final model: $F(3,56)=5.574$, $p=0.002$, $\Delta R^2=18.9$). Children in the high GL groups did better than children in the low GL groups on this test. In the low GL groups girls did better than boys, while in the high GL groups boys did better than girls.

Speed of information processing

Performance on the speed of information processing task was predicted by both the GI and the GL, the interaction between gender and GI, as well as being taller ($F(1,54)=4.928$, $p=0.031$, $F(1,54)=11.199$, $p=0.001$, $F(1,54)=2.126$, $p=0.046$, and $F(1,54)=9.956$, $p=0.003$, respectively; final model: $F(5, 54)=5.026$, $p=0.001$, $\Delta R^2=25.4$). Gender was unrelated to performance on this test ($F(1,54)=0.169$, $p=0.683$). This effectively means that the high GL groups performed better than the low GL groups, and the low GI groups better than the high GI groups; specifically, females in the low GI groups performed better than females in the high GI groups.

Serial sevens

For the serial sevens task, being male, being older and the interaction between GI and GL predicted better performance ($F(1,54)=12.484$, $p=0.001$, $F(1,54)=5.882$, $p=0.019$, and $F(1,54)=5.124$, $p=0.028$, respectively; final model: $F(5,54)=6.670$, $p<0.001$, $\Delta R^2=32.5$). The interaction between GI and GL revealed that performance in the low

GL groups was the same for both the low and the high GI groups, while in the high GL groups performance was better for the low GI compared with the high GI group.

Delayed recall

Finally, height was positively associated, and feeling 'happy' before the CF tests negatively associated with delayed recall performance ($F(1,57)=5.055$, $p=0.028$, and $F(1,57)=6.010$, $p=0.017$, respectively; final model: $F(2,57)=6.644$, $p=0.003$, $\Delta R^2=16.1$).

Overall, the low GI – high GL breakfast was statistically significantly associated with improved performance on the speed of information processing and the serial sevens task. These tasks were rated among the three most difficult tasks by the participants. The addition of covariates in the ANOVA model improved the model for most tests; four out of the seven tests were associated with either GI or GL, or both. Specifically, GI was associated with immediate recall performance (i.e. high GI, better performance), GL with matrices performance (i.e. high GL, better performance), GI and GL with speed of information processing performance (i.e. low GI and high GL, better performance), and GI*GL interaction with serial sevens performance (i.e. in the high GL group only, low GI better performance than high GI). Evidence from the cross-sectional study is consistent with the hypothesis for a GI and GL effect for the speed of information and serial sevens task, and for a GL but not a GI effect for the matrices. On the contrary, evidence is inconsistent with the hypothesis for a GI effect for the immediate word recall. Therefore, it seems that GI or GL or both were associated with performance on the majority of the CF tests used, confirming that this method of classifying participants is appropriate for investigating the effects of the glycaemic potency of breakfast on CF.

4.2 Predicting glycaemic and insulinaemic responses from mixed breakfast meals

4.2.1 Characteristics of the sample

The aim of this study was to investigate the postprandial glycaemic, insulinaemic and cortisol responses over a period of three hours after the ingestion of five breakfast meals differing in their GI and GL. Ten healthy young adults (5 males, 5 females) took part in this study (Table 4.11). Their BMI was within the normal adult BMI range (18.5-25kg/m^2) and all of them were habitual breakfast eaters; five of them reported having breakfast everyday (three females, two males), and five twice or more a week (two females, three males). There were no differences between males and females with regard to height, weight and BMI; males were older than females.

Table 4.11: Descriptive characteristics in ten adults participating in the study, all participants, by gender.

	Females		Males		All participants		
n	5		5		10		
Statistics	Mean	se	Mean	se	Mean	se	p*
Age (y)	21.7	0.6	26.9	1.8	24.3	1.2	*0.024*
Height (cm)	163.5	2.7	174.3	5.5	168.9	3.4	0.129
Weight (kg)	57.7	4.1	70.2	4.7	63.9	3.6	0.082
BMI (kg/m^2)	21.5	1.0	23.0	0.6	22.2	0.6	0.238

*Unpaired t-test between males and females.

Two-tailed significance, $p<0.05$.

None of the participants had any learning disabilities, or any known diseases/ conditions, as assessed by the screening form; no one was taking any medication prescribed by a doctor on a regular basis. None of the participants was on a special diet for medical reasons or was currently on a diet, and all of them considered their weight to be about right. One male reported smoking on average three cigarettes per day. The average time that the participants had their usual breakfast was 08:35 (se=7 min), and they consumed it within 24 min (se=2 min) (as reported in their screening forms). The average time that the participants started their test breakfasts (for all meals) was at 08:55 (se=5 min). There were no statistically significant differences between the time the subjects had their usual breakfast and the time each one of the five breakfast meals were administered (paired t-test). Two of the participants never

exercised (females), four exercised once a week (males), three twice or more a week (females), and one every day (male). Four participants were White (two females, two males, 40%), one male was Pakistani (10%), two females were Chinese (20%), one male was Asian-other (10%), and the final two participants were from other ethnic backgrounds (one Arab female, one mixed White/ Chinese male, 20%).

The general health status of the participants was assessed by their full blood count (FBC), serum ferritin (SF), and serum transferrin receptor (STfR) (Table 4.12). The lower levels observed in red blood cells (RBC), Hb and packed cell volume (PCV or HCT) in females on the last visit, compared with the first, are most likely attributable to the CV of the lab method (see Appendix III.10). Serum Ferritin, STfR, RBC, and Hb were within the normal levels for all participants, confirming that none of the participants was anaemic. As far as the other blood parameters are concerned, white blood cells (WBC), RBC, Hb, PCV, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelet count (PLT), platelet distribution width (PDW), neutrophil granulocytes, lymphocytes, monocytes, eosinophil granulocytes, and basophil granulocytes were all within the normal range for all participants on both the first and the last visit. Though there were blood parameters outside the normal limits none of these were abnormally low or high, indicating that all participants were in good general health.

Table 4.12: General health status as assessed by FBC, Serum Ferritin and Serum Transferrin receptor, for all ten participants by gender.

	First visit				Last visit				Normal range			
	Females		Males		Females		Males		Females	Males	Females	Males
n	5		5		5		5					
	Mean	se	Mean	se	Mean	se	Mean	se			p*	
Serum Ferritin (µg/l)	47.8	14.295	94.2	19.166					15-150	15-200		
Serum Transferrin receptor (µg/dl)	15.9	0.977	15.7	0.589					6.4-25.7	6.4-25.7		
n	5		5		5		5					
WBC (10^9/l)	5.8	0.816	6.8	1.809	5.4	0.256	5.5	0.665	4.0-11.0	4.0-11.0	0.686	0.367
RBC (10^{12}/l)	4.4	0.153	5.1	0.066	4.1	0.132	5.0	0.080	3.8-5.8	4.5-5.8	***0.015***	0.212
Hb (g/dl)	12.8	0.249	14.5	0.463	12.0	0.304	14.5	0.552	11.5-15.5	13.0-16.5	***0.004***	0.312
PCV (l/l)	0.4	0.008	0.4	0.006	0.4	0.011	0.4	0.012	0.37-0.47	0.4-0.540	***0.010***	0.214
MCV (fl)	87.4	2.013	85.5	2.052	87.7	1.926	85.8	2.105	79.0-96.0	79.0-96.0	0.499	0.865
MCH (pg)	29.0	0.712	28.5	1.193	29.0	0.597	29.0	0.928	27.0-32.0	27.0-32.0	0.908	0.353
MCHC (g/dl)	33.2	0.332	33.3	0.642	33.1	0.328	33.8	0.381	31.5-36.0	31.5-36.0	0.554	0.618
RDW%	12.8	0.235	12.7	0.247	12.9	0.331	12.6	0.206	11.6-14.6	11.6-14.6	0.324	0.703
PLT (10^9/l)	250	14.365	239	23.932	259	15.468	253	18.203	150-450	150-450	0.268	0.137
PDW (fl)	13.0	0.433	13.5	0.667	12.38	0.559	12.6	0.643	2-20	2-20	0.100	0.281
Neutrophils (10^9/l)	3.60	0.851	3.97	1.774	2.95	0.369	2.65	0.405	2.5-7.5	2.5-7.5	0.451	0.422
Lymphocytes (10^9/l)	1.68	0.109	2.13	0.401	1.89	0.216	2.11	0.298	1.3-4.0	1.3-4.0	0.288	0.925
Monocytes (10^9/l)	0.38	0.025	0.50	0.088	0.41	0.025	0.48	0.066	0.2-1.0	0.2-1.0	0.279	0.638
Eosinophils (10^9/l)	0.09	0.019	0.21	0.032	0.12	0.017	0.20	0.035	0.0-0.4	0.0-0.4	0.222	0.867
Basophils (10^9/l)	0.02	0.005	0.02	0.005	0.03	0.009	0.02	0.002	0.0-0.1	0.0-0.1	0.477	0.182

* Paired t-test.

Two-tailed significance, $p<0.05$.

4.2.2 Blood Glucose, Insulin and Cortisol

Repeated blood samples of glucose, insulin and cortisol were taken at baseline, 15, 30, 45, 60, 90, 120, 150 and 180 minutes after breakfast. Three different measurements were taken for glucose: capillary whole blood, capillary plasma, and venous plasma (i.e. laboratory method). The mean values (±se) are presented in Table 4.13. Glucose, insulin and cortisol levels were normally distributed; therefore, parametric tests were used. Repeated measures ANOVA was carried out to examine the differences in the physiological measurements at each time-point between the breakfast meals, using as within-subject factors GL and GI, and one between-subject factor, gender; each one of the within-subject factors had two levels, a high and a low. Therefore, two analyses were carried out. **Analysis A** compared meals M1, M2a, M3, M4 (two high GL meals: one low GI (M1) and one high GI (M2a), and two low GL meals: one low GI (M3) and one high GI (M4); M2a had the same GL as M1); and **analysis B** compared meals M1, M2b, M3, M4 (similarly, two high GL meals: one low GI (M1) and one high GI (M2b), and two low GL meals: one low GI (M3) and one high GI (M4); M2b had the same macronutrient composition as M1). The results of both analyses are presented in Table 4.13. Results are distinguished between significant ($p<0.05$) and borderline significant ($0.05\leq p<0.08$). For both sets of analyses there was no effect of order.

The rate of change in the physiological measurements was assessed by computing the 'minus baseline' values for each one of the measurements and carrying out the repeated measures ANOVA, analysis A and analysis B. This could be considered as a more accurate measure of the rate of change related to each one of the five breakfast meals, as it takes into account the baseline variations. The 'minus baseline' values (±se) for capillary whole blood glucose, capillary plasma glucose, venous plasma glucose, insulin and cortisol are presented in Figure 4.4, Figure 4.5, Figure 4.6, Figure 4.7 and Figure 4.8, respectively. For each one of the physiological measurements the repeated measures ANOVA revealed the following:

4.2.2.a Capillary whole blood glucose

❖ **Capillary whole BG (absolute values) (Table 4.13):**

GL main effects: High GL meals were associated with increased BG levels at all time-points. This effect was statistically significant at 30, 45, 60, and 90 minutes after breakfast for both analyses A and B, and at 180 min for analysis B only ($F(1,7)=96.938$, $p<0.001$, $F(1,7)=7.017$, $p=0.033$; ($F(1,7)=25.436$, $p=0.001$, $F(1,7)=14.740$, $p=0.006$; $F(1,8)=19.228$, $p=0.002$, $F(1,8)=15.312$, $p=0.004$; and $F(1,8)=6.647$, $p=0.033$, $F(1,8)=15.537$, $p=0.004$, respectively; analysis B only: borderline $F(1,8)=4.440$, $p=0.068$).

GI main effects: Blood glucose levels appeared to be higher in the low GI compared with the high GI meals up to 45 min after breakfast administration, then lower (60 up to 90 min) and then again higher (120 up to 180 min). The GI effect was only significant for meal analysis A, at 15 min, and then again at 150 min; low GI meals were associated with higher BG levels compared with the high GI meals ($F(1,8)=9.102$, $p=0.017$, borderline significant: $F(1,8)=5.120$, $p=0.053$, respectively).

Gender main effects: There were no significant gender effects for either analysis A or B.

Interactions: There were two borderline significant GI*GL interactions for analysis A at 15 and 180 min, and one for analysis B at 120 min (Analysis A: $F(1,8)=4.971$, $p=0.056$, $F(1,8)=4.859$, $p=0.059$, respectively; analysis B: $F(1,8)=4.543$, $p=0.066$). For analysis A, in the high GL group the low GI meal had higher BG levels than the high GI (M1>M2a), while in the low GL group the high GI meal had higher BG levels than the low GI (M4>M3). For analysis B, in the high GL group the high GI had higher BG levels than the low GI (M2b>M1), while in the low GL group the low GI had higher BG levels than the high GI (M3>M4). There was only one significant GI*gender interaction at 15 min for both analyses A and B ($F(1,8)=27.876$, $p=0.001$, $F(1,8)=9.951$, $p=0.014$, respectively). The GI*gender interaction revealed that in the low GI meals females had higher BG levels than males, while in the high GI meals males had higher BG levels than females. Furthermore, there were two significant GL*gender interactions at 15 and 30 min for analysis A ($F(1,8)=5.632$, $p=0.045$, $F(1,7)=16.860$, $p=0.005$, respectively); and at 15 and 180 min for analysis B ($F(1,8)=7.862$, $p=0.023$, $F(1,8)=6.688$, $p=0.032$, respectively). The GL*gender interaction at 15 min for both analyses A and B showed that in the high GL meals, females had higher BG than males; in the low GL meals males had higher BG than

females. At 30 min for analysis A there were no differences between males and females in the high GL meals; in the low GL meals males had higher BG than females. At 180 min for analysis B females had higher BG levels than males in the high GL meals, but not in the low GL meals (same between males and females).
M2a *vs.* M2b: Paired t-test revealed that there were no differences between meals M2a and M2b at any of the time-points.

❖ **Capillary whole BG ('minus baseline' values) (Figure 4.4):**

GL main effects: In general, there was a trend for high GL meals to be associated with higher BG levels at all time-points compared with the low GL meals. For analysis A, high GL meals compared with low GL meals had statistically significantly higher BG levels at 30, 45, 60, and 90 minutes after breakfast ($F(1,7)=16.185$, $p=0.005$, $F(1,7)=16.726$, $p=0.005$, $F(1,8)=26.120$, $p=0.001$, $F(1,8)=8.227$, $p=0.021$). For analysis B, at 45, 60, and 90 minutes after breakfast the high GL meals were associated with higher BG levels compared with the low GL meals ($F(1,7)=10.797$, $p=0.013$, $F(1,8)=11.658$, $p=0.009$, $F(1,8)=16.871$, $p=0.003$, respectively).
GI main effects: There was a trend for the low GI meals compared with the high GI meals to be associated with higher BG levels up to 45 min, then lower (60 up to 90 min), and then again higher (120 to 180 min). Nonetheless, this effect was not significant at any of the time-points for either analysis A or B.
Gender main effects: There were no significant gender effects for either analysis A or B.
Interactions: For analysis A there were two significant interactions at both 15 (borderline) and 45 min; a GL*GI interaction and a GL*GI*gender interaction (borderline: $F(1,8)=4.531$, $p=0.066$, $F(1,7)=6.377$, $p=0.040$; borderline: $F(1,8)=4.531$, $p=0.066$, $F(1,7)=9.273$, $p=0.019$, respectively). At 15 min the GL*GI interaction revealed that in the high GL group the low GI meal had higher BG levels than the high GI meal (M1>M2a), while in the low GL group the exact opposite was observed (M4>M3). In females, it followed the same pattern, while in males the high GI meal had higher BG levels at both high and low GL groups (M2a>M1 and M4>M3). At 45 min, the GL*GL interaction suggested that in the high GL meals, the high GI meal had higher glucose than the low GI meal (M2a>M1), while in the low GL meals, the exact opposite was observed (M3>M4). Specifically, in females the high GI meals were associated with higher glucose levels both at the high and the low GL meals

compared with the low GI meals. In males, similar pattern as for the GL*GL interaction was observed (M2a>M1 and M3>M4). For analysis B, the same two interactions were observed as for analysis A; at 15 min there was a significant GI*GL and a GI*GL*gender interaction ($F(1,8)=6.261$, $p=0.037$, $F(1,8)=5.893$, $p=0.041$, respectively). In the high GL meals, the low GI meal had higher glucose levels than the high GI meal (M1>M2b), while in the low GL meals the high GI meal had higher glucose levels than the low GI meal (M4>M3). As far as the GI*GL*gender interaction is concerned, in females it was similar to the GI*GL interaction (M1>M2b and M4>M3), while in males the high GI meals were associated with higher glucose levels, either at the high or the low GL meals (M2b>M1 and M4>M3).

M2a *vs.* M2b: There were no statistically significant differences between M2a and M2b at any time-point (paired t-test).

4.2.2.b Capillary plasma glucose

❖ **Capillary plasma BG (absolute values) (Table 4.13):**

GL main effects: There was a trend for the high GL meals to be associated with higher BG levels compared with the low GL meals at all time-points. This effect was significant at 30, 45, 60, 90 and 120 min after breakfast, for both analyses A and B ($F(1,7)=59.221$, $p<0.001$, $F(1,7)=18.636$, $p=0.003$; $F(1,8)=17.768$, $p=0.003$, $F(1,8)=12.290$, $p=0.008$; $F(1,8)=45.433$, $p<0.001$, $F(1,8)=23.900$, $p=0.001$; $F(1,8)=12.152$, $p=0.008$, $F(1,8)=29.536$, $p=0.001$; $F(1,8)=2.970$, $p=0.046$, $F(1,8)=6.005$, $p=0.040$, respectively at each time-point). For analysis A, there was in addition a significant effect at baseline, indicating that the 'minus baseline' levels might be a more reliable measure ($F(1,8)=18.053$, $p=0.003$). For analysis B there was also a borderline significant effect at 180 min (borderline: $F(1,8)=4.366$, $p=0.070$).

GI main effects: There was a trend for the low GI meals to be associated with higher BG levels compared with the high GI meals up to 45 min after breakfast; then from 60 up to 90 min the high GI meals to be associated with higher BG levels compared with the low GI meals; and then again from 120 min to 180 min the low GI meals to be associated with higher BG levels compared with the high GI meals. This effect was significant for analysis A at 150 min and borderline significant for analysis B at 30 min, where the low GI meals compared with the high GI meals were associated with increased BG levels ($F(1,8)=5.484$, $p=0.047$, borderline: $F(1,7)=5.105$, $p=0.058$, respectively).

Gender main effects: There were no significant gender effects for either analysis A or B.

Interactions: For both analyses A and B, there was a similar GL*GI interaction at 15 min ($F(1,8)=7.302$, $p=0.027$; $F(1,8)=12.172$, $p=0.008$, respectively). This interaction revealed that in the high GL meals the low GI meal was associated with higher BG levels than the high GI meal (M1>M2a, and M1>M2b, respectively); while in the low GL meals the high GI meal was associated with higher BG levels compared with the low GI meal (M4>M3).

M2a *vs.* M2b: Paired t-test revealed that there were no differences between M2a and M2b at any time-point.

❖ **Capillary plasma BG ('minus baseline' values) (Figure 4.5):**

GL main effects: As for the absolute values, high GL meals were associated with increased BG levels at all time-points compared with the low GL meals. This effect was significant at 30, 45, 60, and 90 min after breakfast for both analyses A and B, and furthermore at 120 and 150 min for analysis B ($F(1,7)=15.224$, $p=0.006$, $F(1,7)=37.123$, $p<0.001$; $F(1,8)=10.167$, $p=0.013$, $F(1,8)=1.0328$, $p=0.012$; $F(1,8)=48.623$, $p<0.001$, $F(1,8)=23.805$, $p=0.001$; $F(1,8)=8.251$, $p=0.021$, $F(1,8)=40.519$, $p<0.001$, respectively; and for analysis B only: $F(1,8)=6.468$, $p=0.035$, borderline: $F(1,8)=4.309$, $p=0.072$, respectively).

GI main effects: In general, for analysis A there was a tendency for the low GI meals to be associated with increased BG levels compared with the high GI meals, with the exception at 90 min where the high GI meals were associated with higher BG levels. This effect was significant at 30 and 150 min, where the low GI meals had higher glucose levels than the high GI meals ($F(1,7)=5.778$, $p=0.047$, borderline: $F(1,8)=4.919$, $p=0.057$, respectively). For analysis B, there was a tendency for the low GI meals to be associated with higher BG levels up to 45 min after breakfast; then the high GI meals up to 120 min; and between 150-180 min the low GI meals. This effect was borderline significant at 30 min, where the low GI meals were associated with higher BG levels (borderline: $F(1,7)=4.779$, $p=0.072$).

Gender main effects: There were no significant gender effects for either analysis A or B.

Interactions: There was a significant GL*GI interaction at 15 min for both analyses A and B ($F(1,8)=10.056$, $p=0.013$, $F(1,8)=8.245$, $p=0.021$, respectively). This

interaction was similar to the one observed for the absolute BG levels, that is in the high GL meals the low GI meal was associated with higher BG levels than the high GI meal (M1>M2a, and M1>M2b, respectively); while in the low GL meals the high GI meal was associated with higher BG levels compared with the low GI meal (M4>M3). This GL*GI interaction was also observed at 90 and 180 min for analysis A; at 180 min though, in the low GL group the low and high GI meal had similar BG levels (M3≈M4) (borderline: F(1,8)=4.993, p=0.067, borderline: F(1,8)=4.289, p=0.072, respectively). For analysis A, there was also a significant GL*gender interaction at 60 min; in the high GL meals males had higher BG levels than females, while in the low GL meals females had higher BG levels than males (F(1,8)=7.622, p=0.025).

M2a *vs.* M2b: When meals M2a and M2b where compared (paired t-test), M2b had higher BG levels 120 min after breakfast (p=0.004).

4.2.2.c Venous plasma glucose (laboratory method)

❖ **Venous plasma BG (absolute values)** (Table 4.13):

GL main effects: Similar to the capillary BG measurements, there was a trend for high GL meals to be related to higher BG levels compared with the low GL meals. Nonetheless, contrary to the capillary measurements, this trend was up to 90 min after breakfast, as from 120 to 180 min the low GL meals appeared to be associated with higher BG levels. There were few significant GL effects, at 45 and 60 min for analysis A, and at baseline, 30, 45, 60 and 90 min for analysis B, where the high GL meals were associated with higher BG levels (Analysis A: F(1,8)=6.639, p=0.033, F(1,8)=8.589, p=0.019, respectively; analysis B: F(1,8)=5.882, p=0.042, borderline: F(1,8)=5.175, p=0.052, borderline: F(1,8)=5.195, p=0.052, borderline: F(1,8)=4.764, p=0.061, F(1,8)=5.359, p=0.049, respectively).

GI main effects: There was a tendency for the low GI meals to be associated with higher BG levels at all time-points; the only exception was for analysis B, where the high GI meals were associated with higher BG levels at 60 and 90 min after breakfast. This effect was significant only for analysis A at baseline, 15, and 30 min after breakfast, where the low GI meals were associated with higher BG levels compared with the high GI meals (F(1,8)=9.153, p=0.016, F(1,8)=14.813, p=0.005, F(1,8)=7.161, p=0.028, respectively).

Gender main effects: There were no significant gender effects for either analysis A or B.

Interactions: For analysis A, there was a borderline significant GL*GI interaction at 180 min; in the high GL group the low GI meal had higher BG levels than the high GI meal (M1>M2a), while there were no differences between the low and high GI meal in the low GL group (M3≈M4) (borderline: $F(1,8)=4.087$, $p=0.078$). In addition, for analysis A, there was a significant GI*gender interaction at 15 min ($F(1,8)=18.882$, $p=0.002$). Females had higher BG levels than males in the low GI meals; and in the high GI meals males higher than females, where the difference between males and females was more profound. For analysis B, there was a significant GL*gender interaction at 15 min; in the high GL meals females had higher BG levels than males, and in the low GL meals, males higher than females ($F(1,8)=7.758$, $p=0.024$).

M2a *vs.* M2b: There were no differences between meals M2a and M2b at any time-point (paired t-test); just at 90 min, where M2b had borderline significantly higher BG levels than M2a ($p=0.078$).

❖ **Venous plasma BG ('minus baseline' values)** (Figure 4.6):

GL main effects: Similar trend to the absolute BG levels was observed, that is the high GL meals were associated with higher BG levels compared with the low GL meals up to 90 min after breakfast, while from 120 to 180 min the low GL meals were associated with higher BG levels. This effect was significant at 45 and 60 min for analysis A, where the high GL meals were associated with higher BG levels ($F(1,8)=6.642$, $p=0.033$, $F(1,8)=6.710$, $p=0.032$, respectively); and at 180 min for both analysis A and B, where the low GL meals were associated with higher BG levels ($F(1,8)=8.072$, $p=0.022$, $F(1,8)=8.512$, $p=0.019$, respectively).

GI main effects: There were no significant GI effects at any time-point for either analysis A or B.

Gender main effects: There were no significant gender effects for either analysis A or B.

Interactions: For analysis A, there was a significant GI*gender interaction at 15 min, and a significant GL*GI*gender interaction at 120 min ($F(1,8)=6.978$, $p=0.030$, $F(1,8)=5.836$, $p=0.042$, respectively). The GI*gender interaction was similar to the one observed for the absolute levels. This effectively means that females had higher

BG levels than males in the low GI meals; and in the high GI meals males had higher than females. The GL*GI*gender interaction revealed that females in the high GL meals had higher BG levels in the high GI meal compared with the low GI meal (M2a>M1), while in the low GL meals females had higher BG levels in the low GI meal compared with the high GI meal (M3>M4). For males, the exact opposite was observed for both high and low GL meals (M1>M2a and M4>M3). For analysis B, there was a significant GL*GI interaction at 180 min; in the high GL meals the low GI meal had higher BG levels than the high GI meal (M1>M2b), while in the low GL meals the high GI meal had higher BG levels than low GI meal (M4>M3) (F(1,8)=5.521, p=0.047).

M2a *vs.* M2b: There were no statistically significant differences between meals M2a and M2b at any time-point (paired t-test).

4.2.2.d Serum insulin

❖ **Serum insulin (absolute values)** (Table 4.13):

GL main effects: High GL meals were associated with higher insulin levels compared with the low GL meals. This effect was significant at most time-points for both analyses A and B, at 30, 45, 60, 90, 120, 150 and 180 min after breakfast (F(1,8)=10.699, p=0.011, F(1,8)=14.447, p=0.005; F(1,8)=18.681, p=0.003, F(1,8)=23.563, p=0.001; F(1,8)=31.007, p=0.001, F(1, 8)=19.783, p=0.002; F(1,8)=12.465, p=0.008, F(1,8)=27.476, p=0.001; F(1,8)=8.073, p=0.022, F(1,8)=12.072, p=0.008; borderline: F(1,8)=4.993, p=0.056, F(1,8)=6.985, p=0.030; F(1,8)=7.560, p=0.025, F(1,8)=9.252, p=0.016, at each time-point respectively).

GI main effects: For analysis A, there was a trend for the low GI meals to be associated with higher insulin levels at all time-points (with an exception at 120 min where the high GI meals were associated with higher insulin levels compared with the low GI meals). This effect was significant at baseline, 15 and 30 min, where the low GI meals were associated with higher insulin levels compared with the high GI meals (F(1,8)=7.481, p=0.026, borderline: F(1,8)=4.848, p=0.059, F(1,8)=5.859, p=0.042, respectively). For analysis B, there was a tendency for the low GI meals to have higher insulin levels up to 60 min after breakfast, while from 90-180 min the high GI meals to have higher insulin levels. This effect was significant at baseline and 30 min, where the low GI meals were associated with higher insulin levels, and at 120 min

where the high GI meals were associated with higher insulin levels (F(1,8)=5.713, p=0.044, borderline F(1,8)=4.995, p=0.057, F(1,8)=5.557, p=0.046, respectively)

Gender main effects: There were no significant gender effects for either analysis A or B.

Interactions: For analysis A, there was one significant GL*GI interaction at 180 min; in the high GL meals the low GI meal was associated with higher insulin levels compared with the high GI meal (M1>M2a), while there were no differences between low and high GI in the low GL meals (M3≈M4) (F(1,8)=5.634, p=0.045). For analysis B, there was one borderline significant GL*GI interaction at 150 min, where in the high GL group the high GI meal was associated with higher BG compared with the low GI meal (M2b>M1), while in the low GL group there were no differences between the low and the high GI meal (M3≈M4) (borderline: F(1,8)=4.427, p=0.069). Furthermore, there was a significant GI*gender interaction at 15 min for both analyses A and B; in the low GI meals females had higher insulin levels than males, while in the high GI meals there were no differences between the two genders (F(1,8)=6.823, p=0.031, F(1,8)=6.641, p=0.033, respectively).

M2a *vs.* M2b: M2b was associated with higher insulin levels at 150 (borderline: p=0.051) and 180 min (p=0.026) compared with M2a.

❖ **Serum insulin ('minus baseline' values)** (Figure 4.7):

GL main effects: Similar to the absolute values, high GL meals were associated with higher insulin levels, effect which was significant at 30, 45, 60, 90, 120, 150 and 180 min, for both analyses A and B (F(1,8)=10.866, p=0.011, F(1,8)=14.831, p=0.005; F(1,8)=18.874, p=0.002, F(1,8)=25.769, p=0.001; F(1,8)=28.168, p=0.001, F(1,8)=18.813, p=0.002; F(1,8)=11.966, p=0.009, F(1,8)=24.103, p=0.001; F(1,8)=7.574, p=0.025, F(1,8)=10.824, p=0.011; borderline: F(1,8)=4.538, p=0.066, F(1, 8)=6.031, p=0.040; and F(1,8)=6.990, p=0.030, F(1,8)=7.630, p=0.025, at each time-point, respectively).

GI main effects: There were no statistically significant GI effects, but the observed trend was the following: low GI meals compared with the high GI meals were associated with higher insulin levels up to 60 min after breakfast for analysis A, and up to 45 min for analysis B; for the remaining time-points the high GI meals were associated with higher insulin levels compared with the low GI meals for both

analyses. There was one borderline GI effect for analysis A at 30 min, where the low GI meals were associated with higher insulin levels compared with the high GI meals ($F(1,8)=4.317$, $p=0.071$).

Gender main effects: There were no significant gender effects for either analysis A or B.

Interactions: For both analyses A and B, there was a significant GI*gender interaction at 15 min ($F(1,8)=9.013$, $p=0.017$, $F(1,8)=7.874$, $p=0.023$, respectively). This interaction was similar to the one observed for the absolute values; in the low GI meals females had higher insulin levels than males, while in the high GI meals there were no differences between the two genders. Furthermore, for analysis B there was one borderline significant GL*GI interaction at 150 min; in the high GL group the high GI meal was associated with higher insulin levels compared with the low GI meal (M2b>M1), while in the low GL group there were no differences between the low and the high GI meal (M3≈M4) (borderline: $F(1,8)=4.557$, $p=0.065$).

M2a *vs.* M2b: Paired t-test revealed that M2b was associated with higher insulin levels, effect which was borderline significant at 150 (borderline: $p=0.060$) and 180 min (borderline: $p=0.052$) after breakfast.

4.2.2.e Serum cortisol

❖ **Serum cortisol (absolute levels) (Table 4.13):**

GL main effects: There was a tendency for the high GL meals to be associated with higher cortisol levels compared with the low GL meals. Nonetheless, this effect was not significant at any time-point.

GI main effects: There was a tendency for the low GI meals to be associated with higher cortisol levels at all time-points. This effect was significant at 60, 90, 120 and 150 min for analysis A ($F(1,8)=5.892$, $p=0.041$, $F(1,8)=6.497$, $p=0.034$, $F(1,8)=5.850$, $p=0.042$, $F(1,8)=22.492$, $p=0.001$, respectively). For analysis B, the GI effect was significant at 60 and 90 min ($F(1,8)=5.623$, $p=0.045$, $F(1,8)=7.881$, $p=0.023$, respectively).

Gender main effects: There were no significant gender effects for either analysis A or B.

Interactions: There were significant GI*GL interactions at 15, 30, 45, 60, 90, 120 and 150 min for analysis A, and at 15, 30, 45, 60 and 90 min for analysis B (Analysis

A: F(1,8)=6.643, p=0.033, F(1,8)=9.486, p=0.015, F(1,8)=13.588, p=0.006, F(1,8)=12.335, p=0.008, F(1,8)=13.389, p=0.006, borderline: F(1,8)=4.084, p=0.078, borderline: F(1,8)=4.941, p=0.057, respectively; analysis B: F(1,8)=5.818, p=0.042, borderline: F(1,8)=5.342, p=0.050, F(1,8)=7.307, p=0.027, F(1,8)=6.754, p=0.032, F(1,8)=7.909, p=0.023, respectively). This effect revealed that in the high GL meals the low GI meal was associated with increased cortisol levels compared with the high GI meal (M1>M2a and M1>M2b); there were no differences between the low and the high GI meal in the low GL meals (M3≈M4). There were significant GI*GL*gender interactions at 30 and 150 min for analysis A (F(1,8)=7.336, p=0.027, F(1,8)=13.759, p=0.006, respectively); and at 60 and 120 min for analysis B (F(1,8)=5.886, p=0.041, F(1,8)=5.836, p=0.042, respectively). In females, in the high GL meals the low GI meal was associated with higher cortisol levels compared with the high GI meal (M1>M2a and M1>M2b), while in the low GL meals there were no differences between the high and the low GI meal (M3≈M4) (for analysis A: at 30 and 150 min; for analysis B: at 60 min); at 120 min for analysis B, in the low GL meals the high GI meal was associated with higher cortisol levels compared with the low GI meal (M2b>M1). In males, in the high GL meals the low GI meal was associated with higher cortisol levels compared with the high GI meal (M1>M2a and M1>M2b), while in the low GL meals the opposite was observed (M4>M3) (for analysis A: at 30 min; for analysis B: at 60 min); at 150 min for analysis A and at 120 min for analysis B, the low GI meals were associated with higher cortisol levels for both the high and the low GL group (M1>M2a and M1>M2b, M3>M4).

M2a *vs.* M2b: There were no differences between M2a and M2b at any time-point, as assessed by paired t-tests.

❖ **Serum cortisol ('minus baseline' values)** (Figure 4.8)**:**

When the baseline levels were taken into account, there were no significant or borderline significant GL, GI, gender effects or interactions.

Overall, based on the repeated measures ANOVA results, capillary BG measurements yielded more statistically significant results compared with the venous measurements. Out of the two capillary measurements, which have given similar results, it has been decided to use the whole blood measurements for the calculation of iAUC. Besides, this is the recommended site for GI testing (FAO/WHO, 1998). The % difference between capillary whole blood and capillary plasma glucose was -8.1% (sc=1.0), which suggests that capillary whole blood levels were lower by 8% to capillary plasma levels. Paired t-test revealed that the mean values of these measurements differed significantly ($p<0.001$). The observed difference is in line with the suggested 11% difference between whole blood and plasma glucose levels (plasma>whole blood) (D'Orazio *et al.*, 2005).

Table 4.13: Blood glucose, insulin and cortisol levels at baseline, 15, 30, 45, 60, 90, 120, 150 and 180 minutes after breakfast, for all participants by meal.

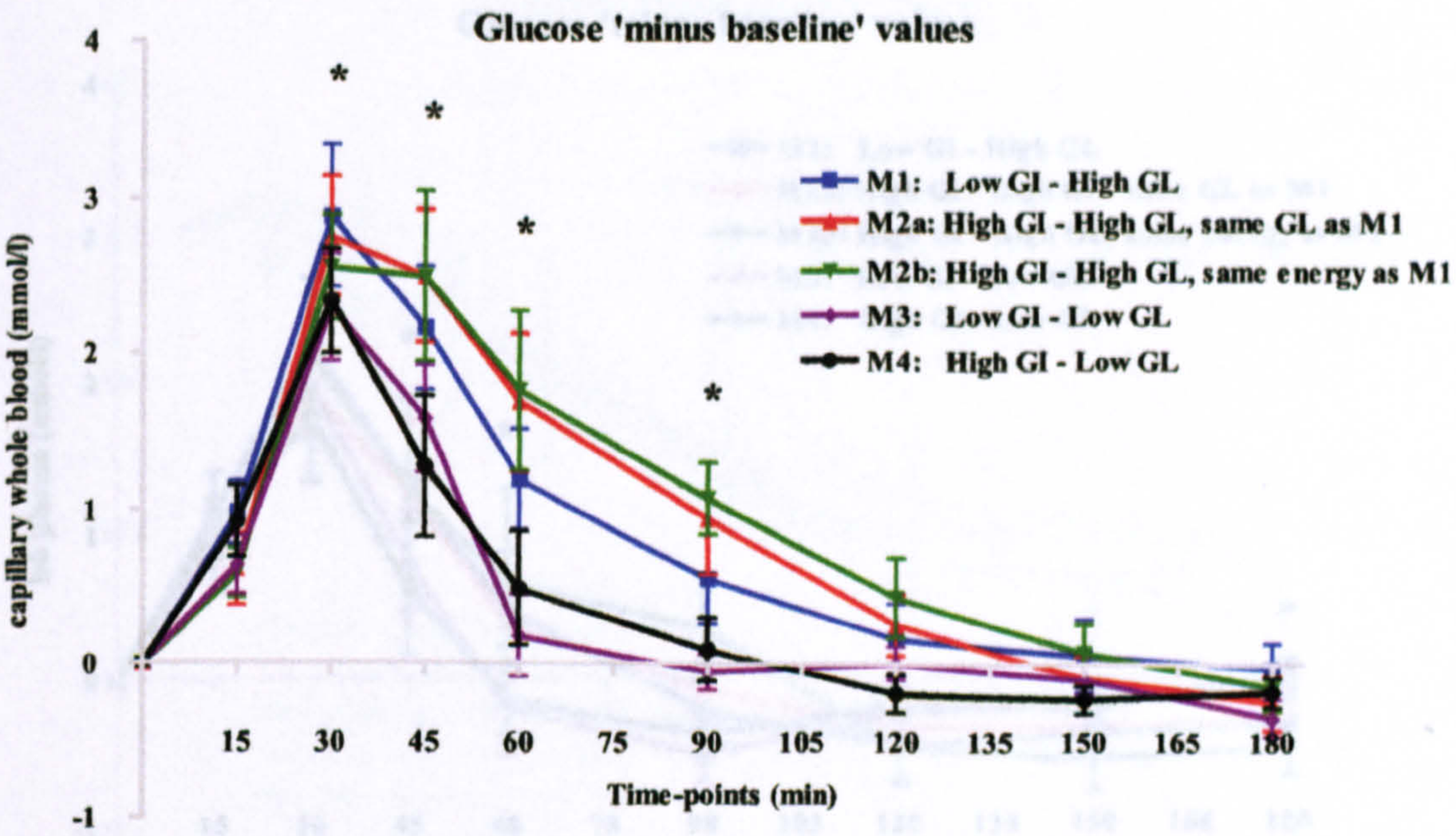
	M1		M2a, iso-GL		M2b, iso-caloric		M3		M4			p*			p†		p‡
	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	GL	GI	GL*GI	GL	GI	GL*GI	
BG CAPILLARY WHOLE (mg/dl)																	
Baseline	4.79	0.18	4.60	0.15	4.71	0.13	4.73	0.13	4.69	0.12	0.912	0.239	0.356	0.616	0.543	0.855	0.485
15 min	5.77	0.26	5.21	0.22	5.30	0.25	5.37	0.24	5.61	0.28	1.000	***0.017***	0.056	0.745	0.239	0.092	0.633
30 min	7.67	0.43	7.35	0.37	7.26	0.38	7.02	0.35	7.02	0.39	***<0.001***	0.276	0.583	***0.033***	0.302	0.398	0.836
45 min	6.95	0.40	7.09	0.42	7.20	0.59	6.30	0.33	5.96	0.45	***0.001***	0.691	0.232	***0.006***	0.318	0.550	0.793
60 min	5.98	0.32	6.28	0.48	6.47	0.55	4.91	0.20	5.18	0.35	***0.002***	0.254	0.960	***0.004***	0.255	0.749	0.728
90 min	5.34	0.28	5.54	0.38	5.78	0.31	4.67	0.12	4.78	0.27	***0.033***	0.415	0.739	***0.004***	0.259	0.287	0.354
120 min	4.95	0.17	4.85	0.23	5.14	0.25	4.73	0.16	4.49	0.16	0.158	0.199	0.413	0.107	0.899	0.066	0.283
150 min	4.84	0.21	4.50	0.15	4.78	0.23	4.62	0.12	4.45	0.15	0.506	0.053	0.568	0.235	0.424	0.595	0.159
180 min	4.76	0.17	4.34	0.13	4.55	0.23	4.37	0.12	4.50	0.18	0.389	0.182	0.059	0.068	0.757	0.238	0.383
BG CAPILLARY PLASMA (mg/dl)																	
Baseline	4.99	0.15	5.24	0.17	4.89	0.09	4.89	0.13	4.85	0.07	***0.003***	0.492	0.430	0.413	0.605	0.830	0.057
15 min	6.68	0.31	5.91	0.30	5.96	0.18	5.78	0.25	6.22	0.26	0.203	0.409	***0.027***	0.161	0.409	***0.008***	0.848
30 min	8.59	0.43	8.06	0.40	7.95	0.44	7.62	0.46	7.51	0.32	***<0.001***	0.211	0.517	***0.003***	0.058	0.333	0.769
45 min	7.48	0.50	7.67	0.40	7.69	0.59	6.87	0.46	6.44	0.50	***0.003***	0.737	0.409	***0.008***	0.720	0.510	0.962
60 min	6.60	0.43	6.73	0.40	6.99	0.57	5.17	0.22	5.22	0.41	***<0.001***	0.706	0.911	***0.001***	0.487	0.723	0.633
90 min	6.19	0.37	6.07	0.39	6.28	0.29	4.63	0.12	4.99	0.21	***0.008***	0.481	0.276	***0.001***	0.352	0.645	0.537
120 min	5.49	0.30	5.34	0.17	5.56	0.20	4.97	0.15	4.77	0.12	***0.046***	0.298	0.863	***0.040***	0.721	0.344	0.219
150 min	5.33	0.25	4.86	0.24	5.18	0.23	5.06	0.10	4.74	0.11	0.366	***0.047***	0.672	***0.041***	0.165	0.666	0.284
180 min	5.41	0.23	4.84	0.25	5.14	0.25	4.91	0.14	4.82	0.13	0.259	0.147	0.181	0.070	0.345	0.668	0.445
BG VENOUS PLASMA (mg/dl)																	
Baseline	4.86	0.10	4.51	0.10	4.74	0.08	4.64	0.11	4.56	0.08	0.290	***0.016***	0.176	***0.042***	0.257	0.826	***0.020***
15 min	5.99	0.28	5.37	0.17	5.57	0.30	5.58	0.16	5.58	0.25	0.362	***0.005***	0.124	0.201	0.320	0.276	0.529
30 min	7.10	0.47	6.43	0.30	6.90	0.49	6.62	0.42	6.32	0.49	0.206	***0.028***	0.552	0.052	0.216	0.816	0.351
45 min	6.18	0.55	5.77	0.51	6.03	0.66	5.44	0.38	5.18	0.52	***0.033***	0.182	0.882	0.052	0.401	0.861	0.695
60 min	5.33	0.39	4.89	0.36	5.40	0.68	4.44	0.18	4.42	0.43	***0.019***	0.285	0.346	0.061	0.943	0.901	0.405

	M1		M2a, iso-GL		M2b, iso-caloric		M3		M4		p*			p†			p‡
BG VENOUS PLASMA (cont)	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	GL	GI	GL*GI	GL	GI	GL*GI	
90 min	4.64	0.27	4.43	0.24	5.08	0.41	4.12	0.13	4.18	0.25	0.084	0.733	0.399	***0.049***	0.389	0.306	0.078
120 min	4.39	0.24	4.16	0.19	4.25	0.21	4.40	0.13	4.37	0.13	0.552	0.280	0.300	0.730	0.421	0.661	0.659
150 min	4.48	0.16	4.16	0.19	4.18	0.22	4.45	0.12	4.34	0.12	0.476	0.188	0.429	0.617	0.214	0.451	0.918
180 min	4.54	0.17	4.22	0.13	4.25	0.19	4.53	0.07	4.58	0.11	0.217	0.154	0.078	0.291	0.222	0.107	0.905
INSULIN (mIU/L)																	
Baseline	9.63	1.45	7.06	0.96	7.50	0.85	7.81	1.02	7.43	0.77	0.276	***0.026***	0.281	0.181	***0.044***	0.299	0.358
15 min	37.96	8.85	25.96	3.87	26.09	4.29	28.62	3.77	25.27	4.61	0.411	0.059	0.192	0.355	0.100	0.248	0.968
30 min	81.93	9.02	61.99	9.26	67.15	8.91	58.03	8.12	49.88	5.72	***0.011***	***0.042***	0.171	***0.005***	0.057	0.325	0.259
45 min	72.51	11.54	56.42	9.71	62.49	8.41	45.35	6.97	39.59	9.22	***0.003***	0.099	0.272	***0.001***	0.104	0.592	0.388
60 min	59.76	10.27	47.60	5.80	57.65	9.64	28.82	5.23	29.51	8.25	***0.001***	0.284	0.269	***0.002***	0.871	0.793	0.291
90 min	44.08	8.51	39.55	11.39	46.28	6.66	16.73	1.76	20.47	5.07	***0.008***	0.951	0.160	***0.001***	0.530	0.811	0.374
120 min	29.56	7.11	29.20	7.98	41.93	9.48	11.57	1.35	14.96	3.32	***0.022***	0.600	0.365	***0.008***	***0.046***	0.167	0.116
150 min	22.20	6.39	20.02	5.49	27.63	6.55	11.33	2.08	10.62	2.14	0.056	0.472	0.718	***0.030***	0.234	0.069	0.051
180 min	17.43	4.10	14.09	3.62	18.67	4.47	8.68	1.20	9.11	1.65	***0.025***	0.153	***0.045***	***0.016***	0.496	0.745	***0.026***
CORTISOL (µg/L)																	
Baseline	548.30	54.22	416.80	52.51	454.50	31.22	463.60	52.42	461.00	43.04	0.524	0.254	0.157	0.340	0.369	0.192	0.397
15 min	575.30	35.79	435.40	44.95	456.30	36.55	474.40	47.47	481.00	54.00	0.487	0.141	***0.033***	0.364	0.217	***0.042***	0.339
30 min	534.40	29.17	389.50	42.26	405.20	37.84	429.00	45.09	431.70	54.03	0.403	0.100	***0.015***	0.365	0.150	0.050	0.568
45 min	486.20	19.19	349.50	32.40	361.80	37.03	376.90	36.44	389.10	46.50	0.256	0.125	***0.006***	0.288	0.116	***0.027***	0.675
60 min	462.90	12.67	327.90	29.10	335.50	40.00	362.70	35.71	370.60	45.81	0.440	***0.041***	***0.008***	0.460	***0.045***	***0.032***	0.780
90 min	380.80	18.69	261.80	26.34	264.00	27.74	298.20	33.73	307.40	37.83	0.611	***0.034***	***0.006***	0.592	***0.023***	***0.023***	0.920
120 min	325.60	30.44	237.30	27.61	251.50	22.43	256.50	30.38	238.10	38.60	0.429	***0.042***	0.078	0.331	0.088	0.281	0.624
150 min	316.30	41.71	219.50	23.64	259.10	25.42	258.90	34.58	227.20	22.53	0.522	***0.001***	0.057	0.204	0.119	0.679	0.277
180 min	281.10	27.80	252.80	27.73	247.00	27.75	238.00	29.87	241.30	28.53	0.255	0.583	0.466	0.335	0.514	0.502	0.832

* Two-way ANOVA: M1, M2a, M3, M4; † Two-way ANOVA: M1, M2b, M3, M4

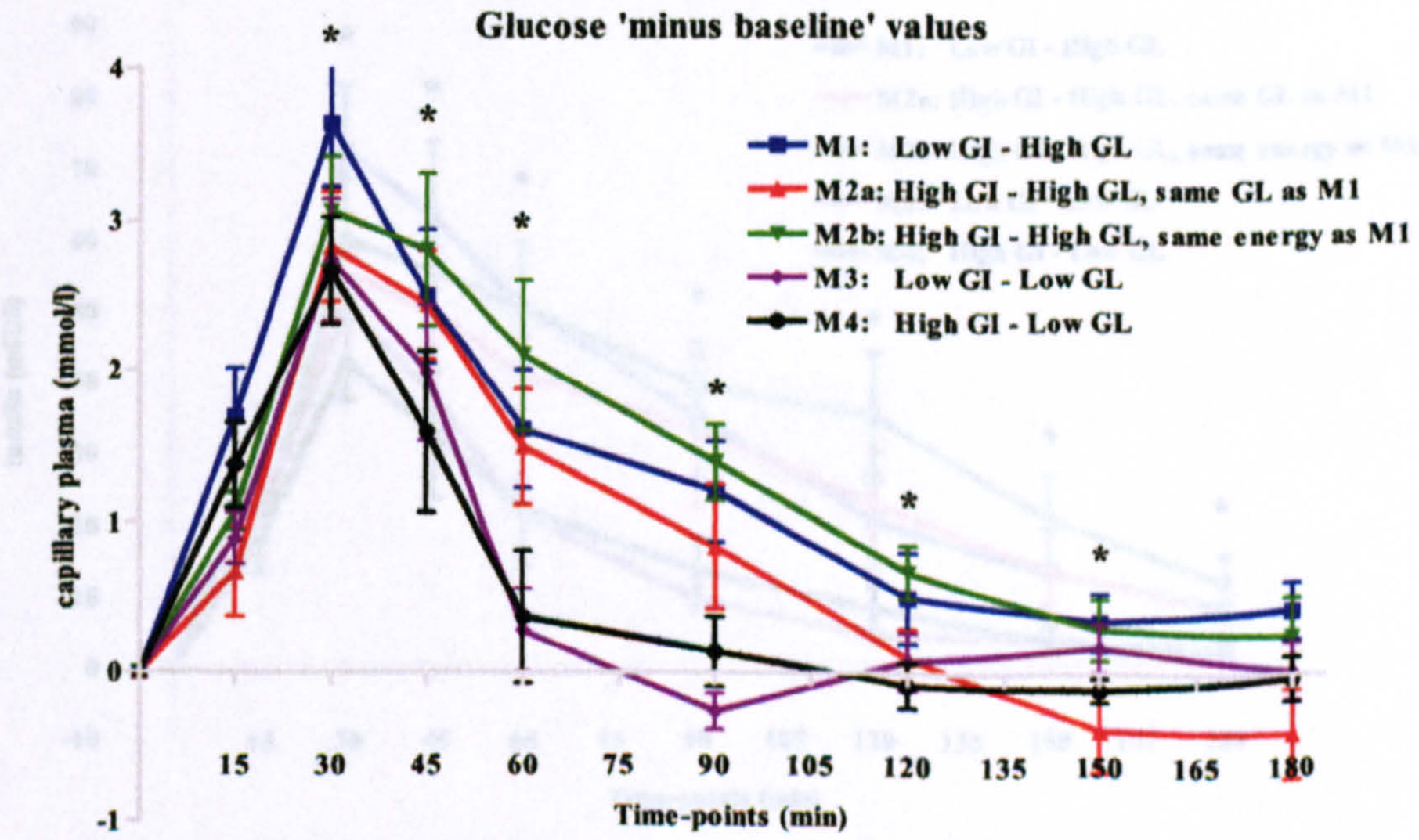
‡ Paired t-test (M2a *vs.* M2b); Two-tailed significance $p<0.05$; Borderline significance $0.05\leq p<0.08$.

Figure 4.4: Capillary whole blood glucose levels ('minus baseline' values) for each one of the five meals at each time-point (mean±se).



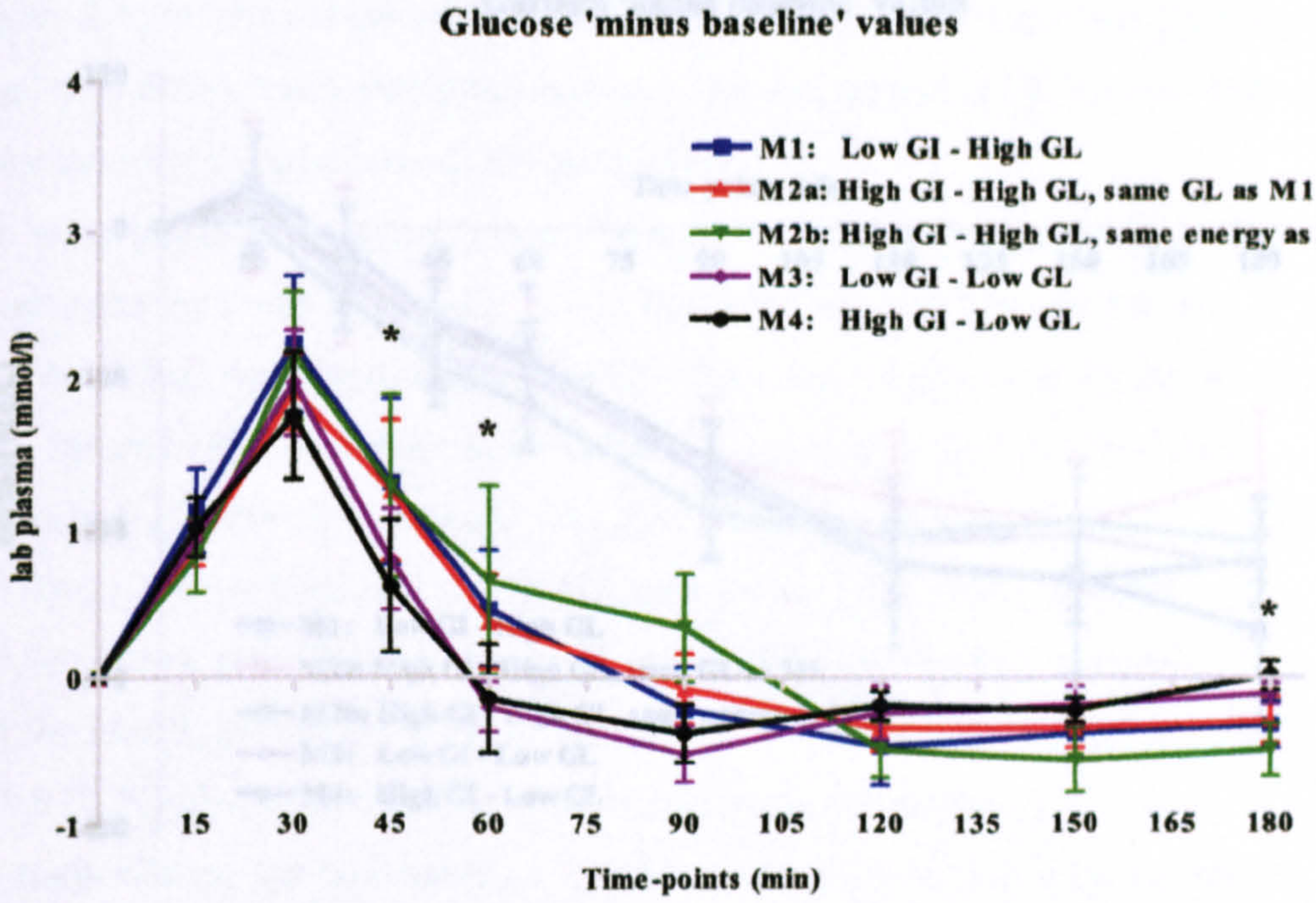
*Statistically significant GL differences; see section 4.2.2.a, page 144 for details.

Figure 4.5: Capillary plasma glucose levels ('minus baseline' values) for each one of the five meals at each time-point (mean±se).



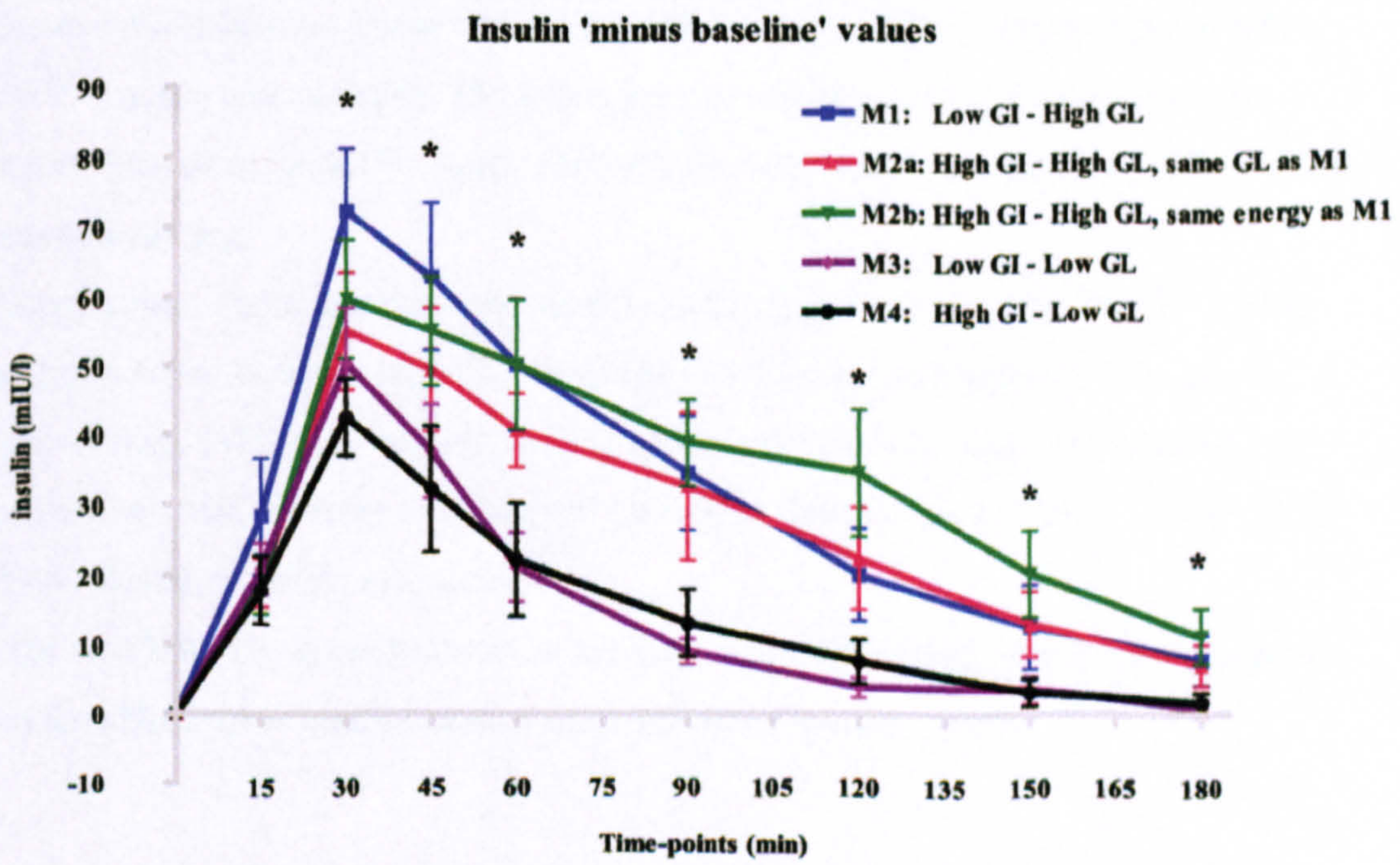
*Statistically significant GL differences; see section 4.2.2.b, page 146 for details.

Figure 4.6: Venous plasma glucose levels ('minus baseline' values) for each one of the five meals at each time-point (mean±se).



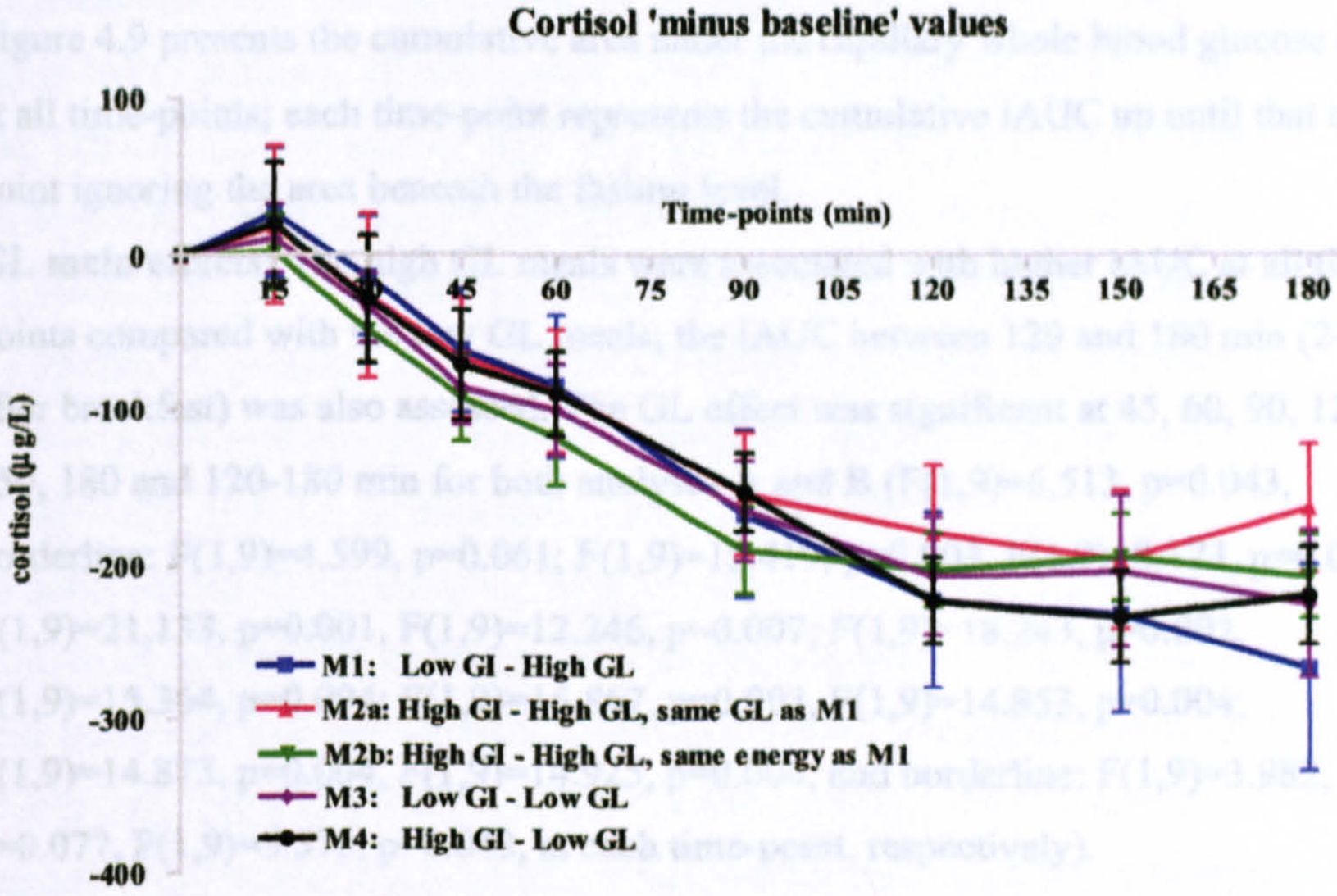
*Statistically significant GL differences; see section 4.2.2.c, page 148 for details.

Figure 4.7: Serum insulin levels ('minus baseline' values) for each one of the five meals at each time-point (mean±se).



*Statistically significant GL differences; see section 4.2.2.d, page 150 for details.

Figure 4.8: Serum cortisol levels ('minus baseline' values) for each one of the five meals at each time-point (mean±se).



4.2.3 Glycaemic and Insulinaemic responses

4.2.3.a iAUC Whole Blood Glucose

Figure 4.9 presents the cumulative area under the capillary whole blood glucose curve at all time-points; each time-point represents the cumulative iAUC up until that time-point ignoring the area beneath the fasting level.

GL main effects: The high GL meals were associated with higher iAUC at all time-points compared with the low GL meals; the iAUC between 120 and 180 min (2-3 h after breakfast) was also assessed. The GL effect was significant at 45, 60, 90, 120, 150, 180 and 120-180 min for both analyses A and B ($F(1,9)=5.513$, $p=0.043$, borderline: $F(1,9)=4.599$, $p=0.061$; $F(1,9)=15.419$, $p=0.003$, $F(1,9)=8.173$, $p=0.019$; $F(1,9)=21.133$, $p=0.001$, $F(1,9)=12.246$, $p=0.007$; $F(1,9)=18.243$, $p=0.002$, $F(1,9)=15.364$, $p=0.004$; $F(1,9)=15.867$, $p=0.003$, $F(1,9)=14.853$, $p=0.004$; $F(1,9)=14.873$, $p=0.004$, $F(1,9)=14.925$, $p=0.004$; and borderline: $F(1,9)=3.982$, $p=0.077$, $F(1,9)=5.572$, $p=0.043$, at each time-point, respectively).

GI main effects: For both analyses A and B, there was a tendency for the low GI meals to be associated with higher iAUC up to 45 min, while from that point onwards the cumulative areas were higher for the high GI meals; between 2-3 h the low GI meals were associated with higher iAUC glucose. The GI effect never reached statistical significance.

Gender main effects: There were no significant gender effects or interactions when iAUC glucose was analysed. Therefore, gender was removed as a factor from the repeated measures ANOVA; unpaired t-test between males and females confirmed the previous finding.

Interactions: There was only one significant GL*GI interactions at 30 min for both analyses A and B; in the high GL meals the low GI meal had higher iAUC than the high GI meal (M1>M2a and M1>M2b), while in the low GL meals the high GI meal had higher iAUC than the low GI meal (M4>M3) (borderline: $F(1,9)=4.336$, $p=0.075$, $F(1,9)=6.159$, $p=0.035$, respectively).

M2a *vs.* M2b: The cumulative areas for M2b were higher than M2a at all time-points, but the effect never reached statistical significance (paired t-test).

4.2.3.b iAUC Insulin

Figure 4.10 presents the cumulative area under the insulin curve at each time-point.

GL main effects: There was a trend for the high GL meals to be associated with higher iAUC insulin at all time-points. This effect was significant at 45, 60, 90, 120, 150, 180 and 120-180 min for both analyses A and B ($F(1,9)=6.282$, $p=0.034$, $F(1,9)=9.449$, $p=0.013$; $F(1,9)=12.303$, $p=0.007$, $F(1,9)=17.882$, $p=0.002$; $F(1,9)=21.545$, $p=0.001$, $F(1,9)=28.511$, $p<0.001$; $F(1,9)=18.995$, $p=0.002$, $F(1,9)=29.314$, $p<0.001$; $F(1,9)=16.660$, $p=0.003$, $F(1,9)=24.727$, $p=0.001$; $F(1,9)=15.100$, $p=0.004$, $F(1,9)=21.263$, $p=0.001$; and $F(1,9)=5.905$, $p=0.038$, $F(1,9)=7.873$, $p=0.021$, at each time-point, respectively).

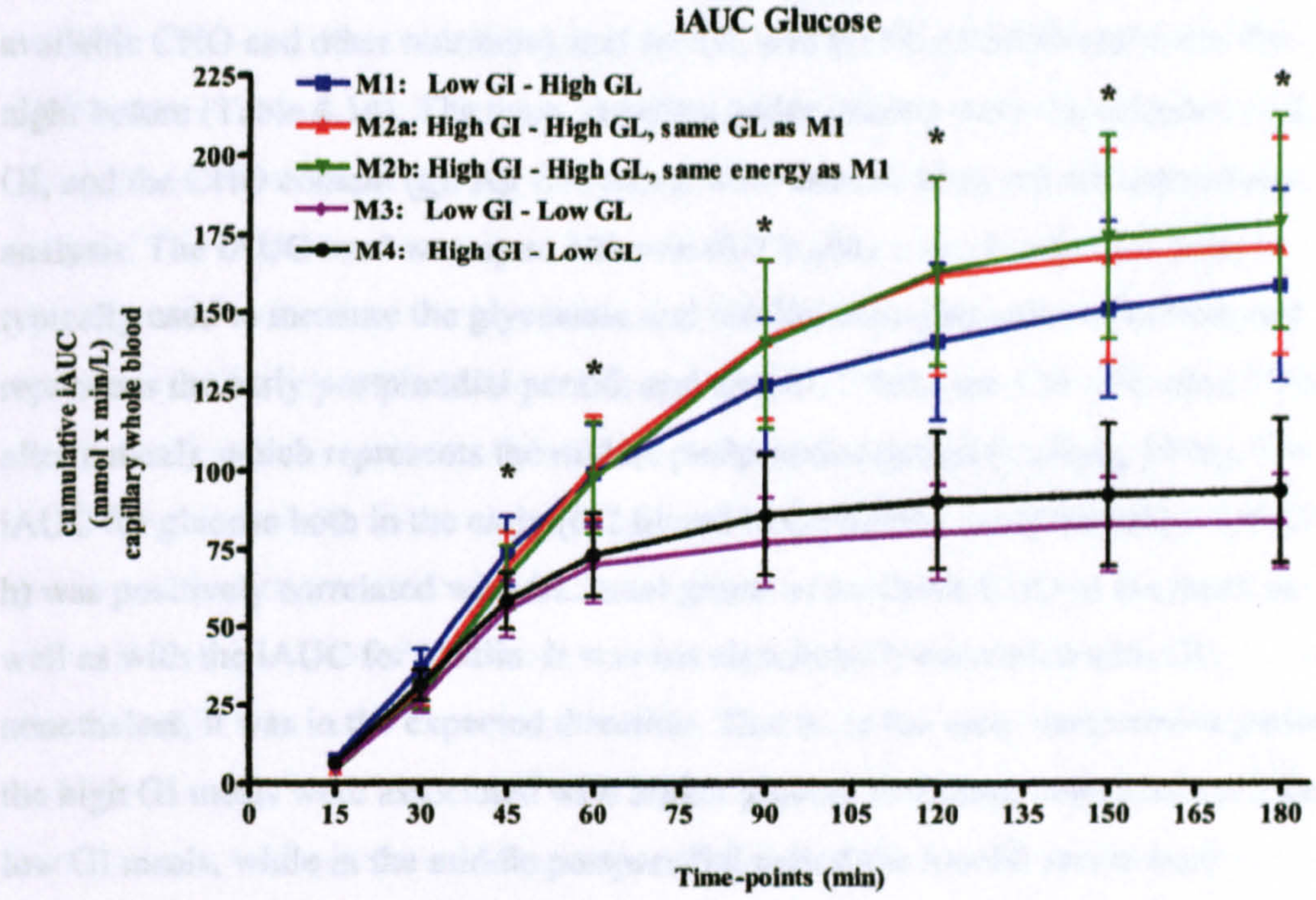
GI main effects: For analysis A, iAUC insulin appeared to be higher in the low GI meals at all time-points, excluding the 120-180 min interval where the high GI meals were associated with higher iAUC insulin. This effect was significant at 30, 45, and 60 min ($F(1,9)=8.437$, $p=0.017$, $F(1,9)=22.336$, $p=0.001$, and $F(1,9)=9.668$, $p=0.013$, respectively). For analysis B, iAUC insulin was higher in the low GI meals up to 90 min; at 120 min the iAUC was about the same for the low and the high GI meals; for the remaining time-points and the 120-180 min interval, the high GI meals had higher iAUC insulin. This GI effect was significant at 30, 45, 60, and 120-180 min (borderline: $F(1,9)=4.443$, $p=0.064$, $F(1,9)=11.580$, $p=0.008$, $F(1,9)=6.450$, $p=0.032$, and $F(1,9)=6.575$, $p=0.030$, respectively).

Gender main effects: Similar to iAUC glucose, there were no gender effects or interactions. Therefore, gender was removed from the analysis.

Interactions: There were no GL*GI interactions, other than at 120-180 min for analysis B; in the high GL group the high GI meal had higher iAUC insulin than the low GI meal (M2b>M1), while there were no differences between the high and low GI meal in the low GL group ($M3 \approx M4$) ($F(1,9)=5.146$, $p=0.049$).

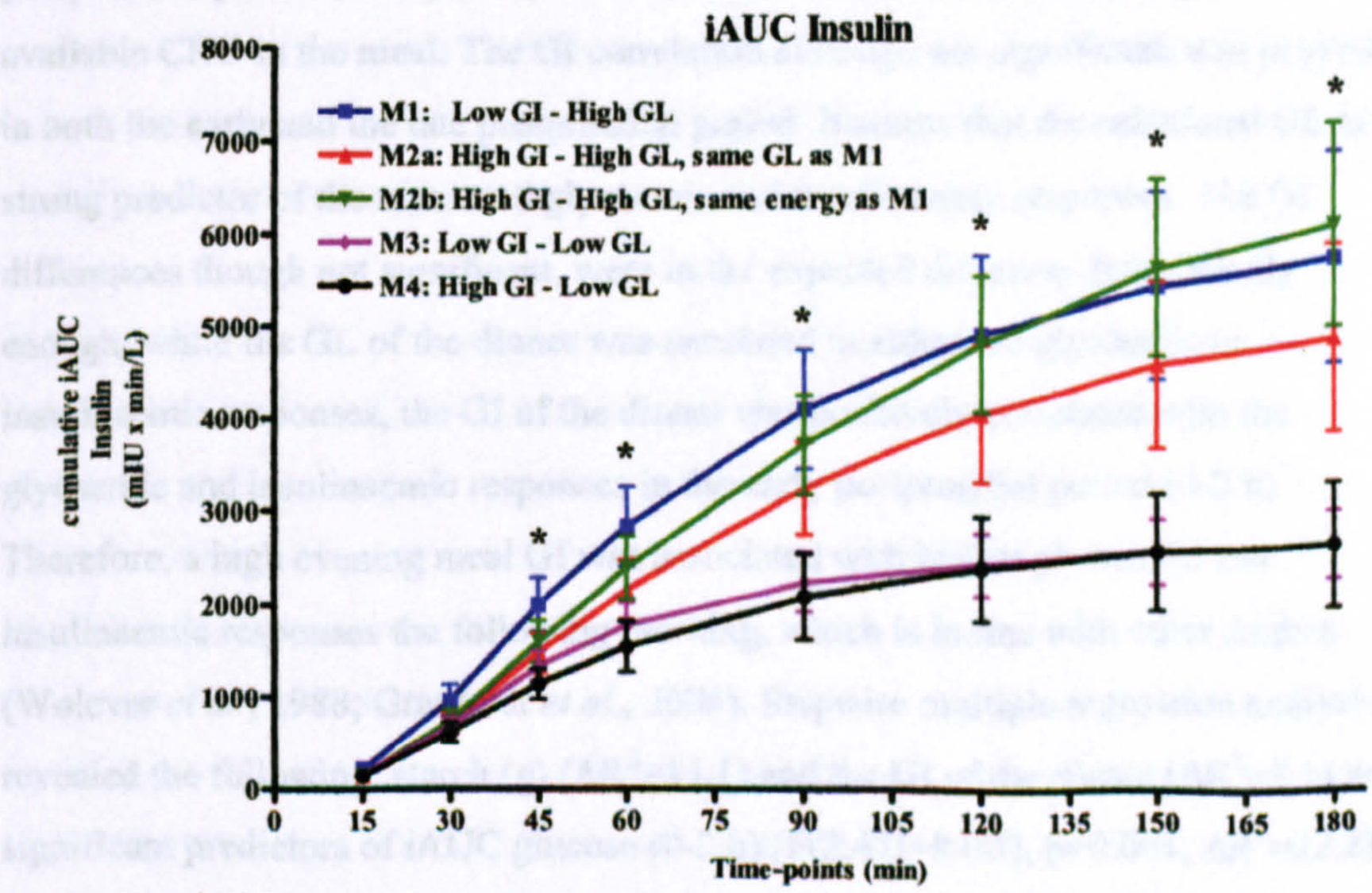
M2a *vs.* M2b: M2b was associated with higher iAUC insulin at all time-points compared with M2a, effect which was borderline significant at 120-180 min ($p=0.065$).

Figure 4.9: Incremental areas under the capillary whole blood glucose curve (mean±se).



*Statistically significant GL differences; see section 4.2.3.a, page 160 for details.

Figure 4.10: Incremental areas under the insulin curve (mean±se).



*Statistically significant GL differences; see section 4.2.3.b, page 161 for details.

Correlation analysis was carried out to reveal linear associations between the iAUC for both whole blood glucose and insulin, and the calculated GL, GI and GL multiplied with GI (GLxGI), the characteristics of the meals (e.g. energy, grams of available CHO and other nutrients), and the GL and the GI of the dinner eaten the night before (Table 4.14). The main variables under interest were the calculated GL, GI, and the CHO content (g). All five meals were used to carry out the correlation analysis. The iAUC used was up to 120 min (0-2 h after a meal), which is what is typically used to measure the glycaemic and insulinaemic responses of a meal, and represents the early postprandial period; and the iAUC between 120-180 min (2-3 h after a meal), which represents the middle postprandial period (Ludwig, 2002). The iAUC for glucose both in the early (0-2 h) and in the middle postprandial period (2-3 h) was positively correlated with GL, total grams of available CHO in the meal, as well as with the iAUC for insulin. It was not significantly correlated with GI; nonetheless, it was in the expected direction. That is, in the early postprandial period the high GI meals were associated with higher glucose responses compared with the low GI meals, while in the middle postprandial period the low GI meals were associated with higher glucose responses compared with the high GI meals.

Similarly, the iAUC for insulin both in the early (0-2 h) and in the middle postprandial period (2-3 h) was positively correlated with GL and total grams of available CHO in the meal. The GI correlation although not significant, was positive in both the early and the late postprandial period. It seems that the calculated GL is a strong predictor of the measured glycaemic and insulinaemic responses. The GI differences though not significant, were in the expected direction. Interestingly enough, while the GL of the dinner was unrelated to either the glycaemic or insulinaemic responses, the GI of the dinner was positively correlated with the glycaemic and insulinaemic responses in the early postprandial period (0-2 h). Therefore, a high evening meal GI was associated with higher glycaemic and insulinaemic responses the following morning, which is in line with other studies (Wolever *et al.*, 1988; Granfeldt *et al.*, 2006). Stepwise multiple regression analysis revealed the following: starch (g) (ΔR^2=15.1) and the GI of the dinner (ΔR^2=7.1) were significant predictors of iAUC glucose (0-2 h) ($F(2,47)=8.002$, $p=0.001$, ΔR^2=22.2); sugar (g) was a significant predictor of iAUC glucose (2-3 h) ($F(1,48)=7.084$, $p=0.011$, ΔR^2=11.0); CHO (g) (ΔR^2=21.7) and the GI of the dinner (ΔR^2=5.8) was a

significant predictor of iAUC insulin (0-2 h) ($F(2,47)=10.273$, $p<0.001$, $\Delta R^2=27.5$); and GL was a significant predictor of iAUC insulin (2-3 h) ($p=0.001$, $\Delta R^2=18.7$).

Overall, from the previous analyses important conclusions can be drawn based on the 'before minus baseline' measures. There were no significant gender effects for glycaemic, insulinaemic and cortisol responses, suggesting that males and females respond similarly to meals differing in their GI and GL. Glycaemic load was a strong predictor of the capillary whole blood glucose responses. High GL meals were associated with higher BG levels at all time-points, effect which was significant at 45, 60, and 90 min after breakfast. Glycaemic index was not significantly associated with BG levels; though the observed trend was that up to 45 min the low GI meals were associated with higher BG levels, from 60-90 min the high GI meals, and from 120-180 min the low GI meals. Similarly, for insulin, high GL meals were associated with higher insulin levels, effect which was significant at all time-points (excluding the 15 min time-point). The GI effect was as before not significant, but up to 45 min the low GI meals were associated with higher BG levels (i.e. as for glucose), and thereafter the high GI meals were associated with higher insulin levels (contrary to glucose for the time-points between 120-180 min). Meal M2b (i.e. same energy as M1) was statistically significantly associated with higher insulin responses compared with M2a (i.e. same GL as M1) at 150 and 180 min after breakfast. As far as cortisol is concerned, there were no meal effects, suggesting that cortisol responses are unrelated to meal administration; the presence of stress would not be expected to have resulted in differences in cortisol responses.

Furthermore, the high GL meals were associated with higher iAUC for glucose and insulin, both in the early (0-2 h) and the middle (2-3 h) postprandial period. Nonetheless, for analysis A, the GL effect was borderline significant in the middle postprandial period for iAUC glucose ($p=0.077$). The GI effects were not significant for iAUC glucose, but were as expected: high GI meals were associated with higher BG levels 0-2 h after breakfast, and low GI meals were associated with higher BG levels 2-3 h after breakfast. For iAUC insulin, in the early postprandial period the low GI meals were associated with higher insulin levels for analysis A, while there were no differences between the GI meals for analysis B (GI effects for either analysis were not significant). In the middle postprandial period, the high GI meals were related to

higher insulin levels, effect which was significant only for analysis B; and M2b had higher insulin levels compared with M2a. Therefore, since analysis B produced more significant results, and M2b differed from M2a, it was concluded that the most appropriate analysis to study the effects of GI and GL on cognition would be analysis B. That is, two high GL meals and two low GL meals that have similar energy and macronutrient composition (but not the high compared with the low GL meals). These four meals (M1, M2b, M3 and M4) were used in the intervention study in children.

Table 4.14: Pearson correlation coefficient between iAUC glucose, iAUC insulin and possible predictors.

	iAUC glucose, 120 min†		iAUC glucose, 120-180 min†		iAUC insulin, 120 min†		iAUC insulin, 120-180 min†	
	Mean	**se**	**Mean**	**se**	**Mean**	**se**	**Mean**	**se**
Total	127.45	11.95	9.92	2.41	3725.54	328.44	681.72	125.48
	Pearson Correlation Coefficients							
GL breakfast*	*0.402*[b]		*0.308*[a]		*0.447*[b]		*0.451*[c]	
GI breakfast*	0.160		-0.029		0.020		0.154	
Carbohydrate (g)	*0.383*[b]		*0.349*[a]		*0.483*[c]		*0.432*[b]	
GIxGL (multiplication)	*0.390*[b]		0.255		*0.389*[b]		*0.436*[b]	
iAUC insulin, 120 min†	*0.673*[c]							
iAUC insulin, 120-180 min†			*0.702*[c]					
Energy (kcal)	*0.361*[a]		*0.356*[a]		*0.482*[c]		*0.417*[b]	
Protein (g)	0.161		*0.291*[a]		*0.343*[a]		*0.285*[a]	
Fat (g)	-0.117		0.113		0.074		-0.066	
Starch (g)	*0.410*[b]		*0.282*[a]		*0.425*[b]		*0.446*[b]	
Sugar (g)	*0.334*[a]		*0.359*[a]		*0.476*[c]		*0.386*[b]	
NMES (g)	*0.353*[a]		*0.358*[a]		*0.482*[c]		*0.396*[b]	
IMS (g)	0.248		0.269		*0.349*[a]		*0.381*[b]	
Glucose (g)	*0.346*[a]		*0.354*[a]		*0.477*[c]		*0.376*[b]	
Fructose (g)	*0.357*[a]		*0.354*[a]		*0.481*[c]		*0.387*[b]	
Sucrose (g)	*0.294*[a]		*0.351*[a]		*0.452*[c]		*0.376*[b]	
Maltose (g)	0.204		*0.307*[a]		*0.383*[b]		0.236	
Lactose (g)	0.026		-0.056		-0.057		0.116	
NSP (g)	0.000		0.181		0.186		0.014	
GL dinner*	0.000		-0.087		-0.054		-0.023	
GI dinner*	*0.357*[a]		0.129		*0.327*[a]		0.193	

* Glycaemic Index (GI) and Glycaemic Load (GL) values corresponding to breakfast meals or evening meals (reference food: glucose).

† iAUC: cumulative area under the glucose or insulin curve, ignoring the area beneath the fasting level.

Pearson correlation coefficients, $p<0.05^{a}$, $p<0.01^{b}$, $p<0.001^{c}$.

4.3 Intervention study in school children

4.3.1 Characteristics of the sample

In brief, the purpose of this dietary intervention trial was to investigate whether the four breakfast meals that differ in their GI and GL (2x2 grid), produce differences in subsequent cognitive function and mood. To test all four breakfast meals (two high GL, and two low GL meals), participants were matched and allocated either to the high or the low GL group (in each group there was one low and one high GI meal). The descriptive characteristics of all 74 children, and of the children allocated to the two GL groups, high and low, are presented in Table 4.15.

Out of the 74 children, 64 were exact matches (32 pairs), and out of the remaining ten pupils, seven were in the low and three were in the high GL group (see methods 3.3.1, page 94, for details of allocation). There were no statistically significant differences in the distribution of males and females between the two GL groups ($\chi^2(1)=0.054$, $p=0.816$). With regard to the total sample of 74 children, the majority was from year 8 (n=40, 54.1%), followed by year 7 (n=30, 40.5%), and year 9 (n=4, 5.4%). There were no differences in the distribution of class year between the two GL groups ($\chi^2(2)=0.184$, $p=0.912$). Thirty two children (43.2%) were White, 25 (33.8%) were Black, nine (12.2%) were Asian, and eight (10.8%) were from other ethnic backgrounds (mixed races). There were no statistically significant differences in the distribution of ethnic groups between the two GL groups ($\chi^2(2)=0.203$, $p=0.903$). Girls were heavier ($p=0.006$) and had a higher BMI ($p=0.001$) than boys. There were no differences with regard to age, height, weight and BMI, when the two GL groups were considered, suggesting that these were well matched. Based on the BMI-for-age cut-off points (WHO, 2007), 67 students (90.5%) had normal BMI, while only seven (9.5%) were overweight; a higher percentage of girls were overweight (n=6, 16.2%), when compared with the boys (n=1, 2.7%). There were no differences in the distribution of BMI-for-age z-scores between the two GL groups ($\chi^2(1)=0.061$, $p=0.805$). None of the participants had low height-for-age (i.e. stunting), and none of the participants was on a diet. Twenty (54%) girls had started their periods; these observations were too few to consider stage of menstrual cycle as a potential confounder. Similarly, only three (4%) participants reported having caffeine (in the

form of tea or coffee, p 365) regularly, that is twice or more a week. Therefore, any caffeine withdrawal symptoms would not be expected to take place or have an effect on performance levels. Sixty seven participants (90.5%) had normal Hb levels, while four males and three females had Hb<120g/l (the cut-off point for anaemia); the Hb range was 100-150g/l. There were no differences in the distribution of Hb (low, normal) between the two GL groups ($\chi^2(1)=1.088$, $p=0.297$). With regard to usual breakfast consumption, as assessed by the parents' screening form, 53 children (71.6%) reported having breakfast everyday, 17 (23%) twice or more a week, and four (5.4%) once a week. A higher percentage of boys than girls (n= 31, 83.8% *vs.* n=22, 59.5%) had breakfast everyday, which is consistent with the results from the cross-sectional study. Within the two GL groups, low and high, the number of students having breakfast everyday were 31 *vs.* 22 (79.5% *vs.* 62.9%), twice or more a week five *vs.* 12 (12.8% *vs.* 34.3%), and once a week three *vs.* one (7.7% *vs.* 2.9%), respectively. There were no differences in the distribution of how often the participants had breakfast between the low and the high GL groups ($\chi^2(2)=5.210$, $p=0.074$).

Socio-economic class and level of education of the household was measured as the highest of that of the mother or the father. Most parents/ guardians were in AC 2 ('lower managerial and professional occupations') (n=24, 36.9%), AC 1.2 ('higher professional occupations') (n=8, 12.3%) and AC 6 ('semi-routine occupations') (n=8, 12.3%). Similarly, for the low and high GL groups, the majority of the parents were in AC 2 (n=14 *vs.* n=10, 42.4% *vs.* 31.3%, respectively). Differences in AC grouping between the two GL groups were assessed using the chi-squared test. In order to avoid values for E<1 in the chi-square analysis, AC subgroups were combined as follows: 'higher managerial' (ACs 1.1 and 1.2; n=11); 'lower managerial and professional occupations' (AC 2; n=24); 'skilled and technical' (ACs 3, 4 and 5; n=11); 'routine occupations' (ACs 6 and 7; n=12); and 'never worked and long-term unemployed' (AC 8; n=7). There were no statistically significant differences in the distribution of AC between the two GL groups ($\chi^2(4)=6.921$, $p=0.140$). With regard to the level of education, most parents had a higher degree (n=15, 21.7%) or a degree (n=18, 26.1%); within the low GL group the majority had a higher degree (n=11, 30.6%), and in the high GL a degree (n=10, 30.3%). There were no statistically significant

differences in the distribution of the level of education between the two GL groups ($\chi^2(4)=6.053$, $p=0.195$).

Therefore, the two GL groups, high and low, were very well matched: there were no differences in age, height, weight, BMI or Hb between the participants in the two GL groups (un-paired t-test), or in the distribution of males and females, year class, BMI-for-age z-scores, Hb (low, normal), usual breakfast consumption, ethnic group, AC and level of education between the two GL groups (chi-squared test).

Table 4.15: Descriptive characteristics in 74 children participating in the study, all children, and in the two GL groups, by gender.

	GENDER				ALL CHILDREN		GL GROUPS									
							HIGH						LOW			
	Female		Male				Female		Male		Total		Female		Male		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
	37	100	37	100	74	100	17	100	18	100	35	100	20	100	19	100	39	100
Year																		
•7	13	35.1	17	45.9	30	40.5	7	41.2	8	44.4	15	42.9	6	30.0	9	47.4	15	38.5
•8	23	62.2	17	45.9	40	54.1	10	58.8	8	44.4	18	51.4	13	65.0	9	47.4	22	56.4
•9	1	2.7	3	8.1	4	5.4	0	0.0	2	11.1	2	5.7	1	5.0	1	5.3	2	5.1
Ethnic group																		
• White	15	40.5	17	45.9	32	43.2	8	47.1	8	44.4	16	45.7	7	35.0	9	47.4	16	41.0
• Black	14	37.8	11	29.7	25	33.8	6	35.3	5	27.8	11	31.4	8	40.0	6	31.6	14	35.9
• Asian	5	13.5	4	10.8	9	12.2	1	5.9	1	5.6	2	5.7	4	20.0	3	15.8	7	17.9
• Mixed races	3	8.1	5	13.5	8	10.8	2	11.8	4	22.2	6	17.1	1	5.0	1	5.3	2	5.1
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
ACs**	33	100	32	100	65	100	17	100	16	100	33	100	16	100	16	100	32	100
• AC 1.1	3	9.1	0	0.0	3	4.6	2	11.8	0	0.0	2	6.1	1	6.3	0	0.0	1	3.1
• AC 1.2	2	6.1	6	18.8	8	12.3	2	11.8	1	6.3	3	9.1	0	0.0	5	31.3	5	15.6
• AC 2	11	33.3	13	40.6	24	36.9	6	35.3	8	50.0	14	42.4	5	31.3	5	31.3	10	31.3
• AC 3	1	3.0	3	9.4	4	6.2	1	5.9	2	12.5	3	9.1	0	0.0	1	6.3	1	3.1
• AC 4	2	6.1	3	9.4	5	7.7	2	11.8	2	12.5	4	12.1	0	0.0	1	6.3	1	3.1
• AC 5	0	0.0	2	6.3	2	3.1	0	0.0	1	6.3	1	3.0	0	0.0	1	6.3	1	3.1
• AC 6	7	21.2	1	3.1	8	12.3	3	17.6	1	6.3	4	12.1	4	25.0	0	0.0	4	12.5
• AC 7	3	9.1	1	3.1	4	6.2	1	5.9	0	0.0	1	3.0	2	12.5	1	6.3	3	9.4
• AC 8	4	12.1	3	9.4	7	10.8	0	0.0	1	6.3	1	3.0	4	25.0	2	12.5	6	18.8

	GENDER					ALL CHILDREN		GL GROUPS											
								HIGH						LOW					
	Female		Male					Female		Male		Total		Female		Male		Total	
	n	%	n	%		n	%	n	%	n	%	n	%	n	%	n	%	n	%
Education	33	100	36	100		69	100	15	100	18	100	33	100	18	100	18	100	36	100
• Higher degree	10	30.3	5	13.9		15	21.7	2	13.3	2	11.1	4	12.1	8	44.4	3	16.7	11	30.6
• Degree	5	15.2	13	36.1		18	26.1	1	6.7	9	50.0	10	30.3	4	22.2	4	22.2	8	22.2
• A Level	4	12.1	8	22.2		12	17.4	3	20.0	2	11.1	5	15.2	1	5.6	6	33.3	7	19.4
• GCSE	6	18.2	7	19.4		13	18.8	3	20.0	3	16.7	6	18.2	3	16.7	4	22.2	7	19.4
• Other	8	24.2	3	8.3		11	15.9	6	40.0	2	11.1	8	24.2	2	11.1	1	5.6	3	8.3
	Mean	se	Mean	se	p*	Mean	se	Mean	Se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	p*
Age (y)	12.7	0.1	12.6	0.1	0.467	12.6	0.1	12.6	0.1	12.7	0.2	12.6	0.1	12.7	0.1	12.5	0.1	12.6	0.1	0.993
Height (cm)	157.6	1.1	156.0	1.4	0.356	156.8	0.9	156.7	1.4	155.5	1.8	156.1	1.1	158.3	1.6	156.3	2.1	157.3	1.3	0.482
Weight (kg)	50.8	1.3	45.6	1.2	***0.006***	48.2	1.0	49.2	1.9	44.6	1.7	46.8	1.3	52.1	1.9	46.5	1.8	49.4	1.4	0.183
BMI (kg/m^2)	20.4	0.4	18.6	0.3	***0.001***	19.5	0.3	19.9	0.6	18.3	0.4	19.1	0.4	20.8	0.6	18.9	0.4	19.9	0.4	0.170
Hb (g/l)	128.6	1.4	130.4	1.3	0.347	129.5	0.9	130.0	2.7	131.4	1.9	130.7	1.6	127.4	1.2	129.4	1.8	128.4	1.1	0.224

* Un-paired t-test.

** Analytic classes (ACs): AC 1.1: Large employers and higher managerial occupations; AC 1.2: Higher professional occupations; AC 2: Lower managerial and professional occupations; AC 3: Intermediate occupations; AC 4: Small employers and own account workers; AC 5: Lower supervisory and technical occupations; AC 6: Semi-routine occupations; AC 7: Routine occupations; AC 8: Never worked before and long term unemployed.

Two-tailed significance, $p<0.05$.

4.3.2 Blood Glucose, Salivary Cortisol and breakfast meals

The mean values of BG and cortisol levels taken at baseline, before and after the CF tests, as well as their differences ('before minus baseline', 'after minus baseline', 'after minus before') by gender, and in all children and in the four GI – GL groups are presented in Table 4.16 and Table 4.17, respectively. Baseline cortisol levels taken at baseline on the non-testing day ('stress-free') are also presented. Glucose and cortisol levels were normally distributed; therefore, parametric tests were used. Un-paired t-tests revealed that there were no differences between females and males in any of the physiological measurements, with the exception of the cortisol difference during the CF testing ('after minus before'), which decreased on average in females and increased in males. There were no differences in the time (AM) glucose and cortisol levels were taken at baseline, before and after the CF tests, for all four meals. Cortisol at baseline on the non-testing day was taken on average 6 min 43 sec (se=01:27) later than cortisol on the first visit, and 6 min 13 sec (se=01:40) later than cortisol on the second visit (paired t-test: $p<0.001$ for both). Paired t-tests showed that there were no differences between baseline cortisol levels on the non-testing day and either baseline cortisol on the first visit ($p=0.188$) or baseline cortisol on the second visit ($p=0.988$); similarly, there were no differences between baseline cortisol on the first and on the second visit ($p=0.151$). Table 4.17 shows that there were not any differences in the time cortisol and glucose values were taken between the four GI and GL groups (one-way ANOVA). On average, glucose before the tests was taken 92.3 min (se=0.3), and glucose after the tests 146.5 min (se=1.0) after breakfast commenced (time zero). Similarly, for cortisol before and after the CF tests, the average time was 89.8 min (se=0.4) and 142.9 min (1.0), respectively. Figure 4.11 and Figure 4.13 depict the glucose and cortisol levels at these time-points, respectively. The whole blood glucose and serum cortisol levels at the relevant time-points from the adult study are presented in Figure 4.12 and Figure 4.14, respectively (see discussion 5.3, page 201). The previous findings suggest that the entire testing procedure, both the screening and the testing days, was accurately timed. Therefore, any differences due to variations in circadian rhythms should not be expected. Furthermore, there were not any differences in baseline cortisol levels between all three visits; this suggests that participants were not stressed at baseline on the testing days more than any other 'stress-free' day. Finally, the salivary cortisol was accurately taken on average at 89.8

min, which is when the testing should have started (i.e. 90 min after breakfast), suggesting that the protocol was accurately timed and closely adhered to.

Repeated measures ANOVA (GI within-subject factor, GL between-subject factor) was carried out to assess the effects of GI, GL and their interaction on glucose and cortisol on both their absolute levels and their differences. The differences were calculated in order to assess the effect of the meals on the degree of change, which therefore takes into account the baseline variations, rather than the observed values *per se*. There were no statistically significant main gender effects or interactions; only for the 'after minus before' measure there was one significant main effect of gender as a between-subject factor (males had higher cortisol levels than females). Since, there was only one significant effect of gender for all measures, it can be argued that there were no differences in either glucose or cortisol levels between males and females. What is more, the order of the meal administration did not have an effect on either glucose or cortisol measures.

4.3.2.a. Glucose levels and GI and GL (Figure 4.11):

There was a tendency for the high GI and high GL meals to be associated with higher BG levels at all time-points compared with the low GI and low GL meals, respectively. At baseline, there was a significant GI*GL interaction; in the low GL group the low GI meal was associated with higher BG compared with the high GI meal (M3>M4), while there were no differences between the low and high GI meal (M1≈M2) in the high GL group. Nonetheless, as this effect was at baseline it does not reflect a meal effect, and furthermore suggests that the 'minus baseline' values would potentially provide a better estimate of any meal (i.e. GI and GL) effects. There was a significant GL effect for BG levels both before and after the CF tests; the high GL meals were associated with higher BG levels compared with the low GL meals ($F(1,72)=15.730$, $p<0.001$, $F(1,72)=12.636$, $p=0.001$, respectively).

When the differences where considered, 'before minus baseline' BG levels were borderline significantly higher in the high GI meals compared with the low GI meals ($F(1,72=3.837$, $p=0.054$), and higher in the high GL meals compared with the low GL meals ($F(1,72)=16.747$, $p<0.001$). The 'after minus baseline' BG levels were

statistically significantly higher in the high GL meals compared with the low GL meals ($F(1,72)=15.813$, $p<0.001$). There were no statistically significant effects when the 'after minus before' values were considered. These findings confirm that the breakfast meals selected produced differences in their glycaemic responses, due to differences in GI and GL, both before the tests (i.e. after the meal) and after the tests (i.e. after the meal and the CF tests). The latter represents the combined effects of both the meals administered and the experimental testing on BG levels (if any), which can not be statistically disentangled.

4.3.2.b. Cortisol levels and GI and GL (Figure 4.13):

At baseline there was a significant GI effect on cortisol levels ($F(1,70)=4.432$, $p=0.039$); the low GI meals were associated with higher cortisol levels at baseline, compared with the high GI meals. Nonetheless, similar to glucose levels, as measured at baseline it does not reflect a meal effect; and it also suggests that the 'minus baseline' levels are a better measure. Before and after the CF tests there was a tendency for the high GL and high GI meals to be associated with higher cortisol levels compared with the low GL and low GI meals, as observed for the BG levels. The GI effect was borderline statistically significant for the cortisol levels after the CF tests ($F(1,70)=0.057$, $p=0.057$). When the differences were considered, there was a tendency for the high GL and the low GI meals to be associated with a bigger drop in cortisol levels when compared with the low GL and the high GI meals. There were no significant main GL effects or GI*GL interactions. The GI effect was statistically significant when the 'before minus baseline' ($F(1,70)=4.805$, $p=0.032$) and the 'after minus baseline' values ($F(1,69)=9.188$, $p=0.003$) were considered. Since the intervention study in adults revealed that these meals did not differ in their cortisol responses (when the 'minus baseline' values were considered), these findings suggest that stress was present, which differed according to the GI of the meals.

Table 4.16: Blood glucose and salivary cortisol levels, and time these were taken, in 74 children participating in the study by gender.

	Gender				
	Females		Males		
n	37		37		
	Mean	se	Mean	se	p†
Time of breakfast (AM)	08:44:38	00:00:54	08:47:23	00:01:12	0.251
Time fp baseline (AM) ‡	08:33:18	00:00:52	08:32:38	00:00:56	0.605
BG baseline (mmol/L)	5.0	0.0	5.1	0.0	0.291
Time fp 'before' (AM) ‡	10:19:07	00:00:54	10:18:29	00:01:07	0.660
BG 'before' (mmol/L)	5.2	0.1	5.3	0.1	0.207
Time fp 'after' (AM) ‡	11:11:59	00:01:33	11:14:04	00:01:57	0.403
BG 'after' (mmol/L)	5.1	0.1	5.2	0.1	0.606
BG 'before-baseline' (mmol/L)	0.2	0.1	0.2	0.1	0.467
BG 'after-baseline' (mmol/L)	0.1	0.1	0.1	0.1	0.824
BG 'after-before' (mmol/L)	-0.1	0.1	-0.2	0.1	0.352
Time cortisol baseline* (AM)	08:34:00	00:02:00	08:30:52	00:01:58	0.268
Cortisol baseline* (ng/mL)	5.3	0.4	4.8	0.3	0.299
Time cortisol baseline (AM)	08:26:20	00:00:47	08:25:14	00:00:54	0.235
Cortisol baseline (ng/mL)	4.9	0.2	4.7	0.2	0.549
Time cortisol 'before' (AM)	10:16:02	00:01:09	10:16:38	00:01:15	0.726
Cortisol 'before' (ng/mL)	3.8	0.2	3.6	0.1	0.138
Time cortisol 'after' (AM)	11:08:47	00:01:28	11:10:03	00:01:52	0.597
Cortisol 'after' (ng/mL)	3.4	0.1	3.7	0.1	0.109
Cortisol 'before-baseline' (ng/mL)	-1.0	0.2	-1.2	0.1	0.568
Cortisol 'after-baseline' (ng/mL)	-1.5	0.2	-1.0	0.1	0.079
Cortisol 'after-before' (ng/mL)	-0.4	0.1	0.2	0.1	***<0.001***

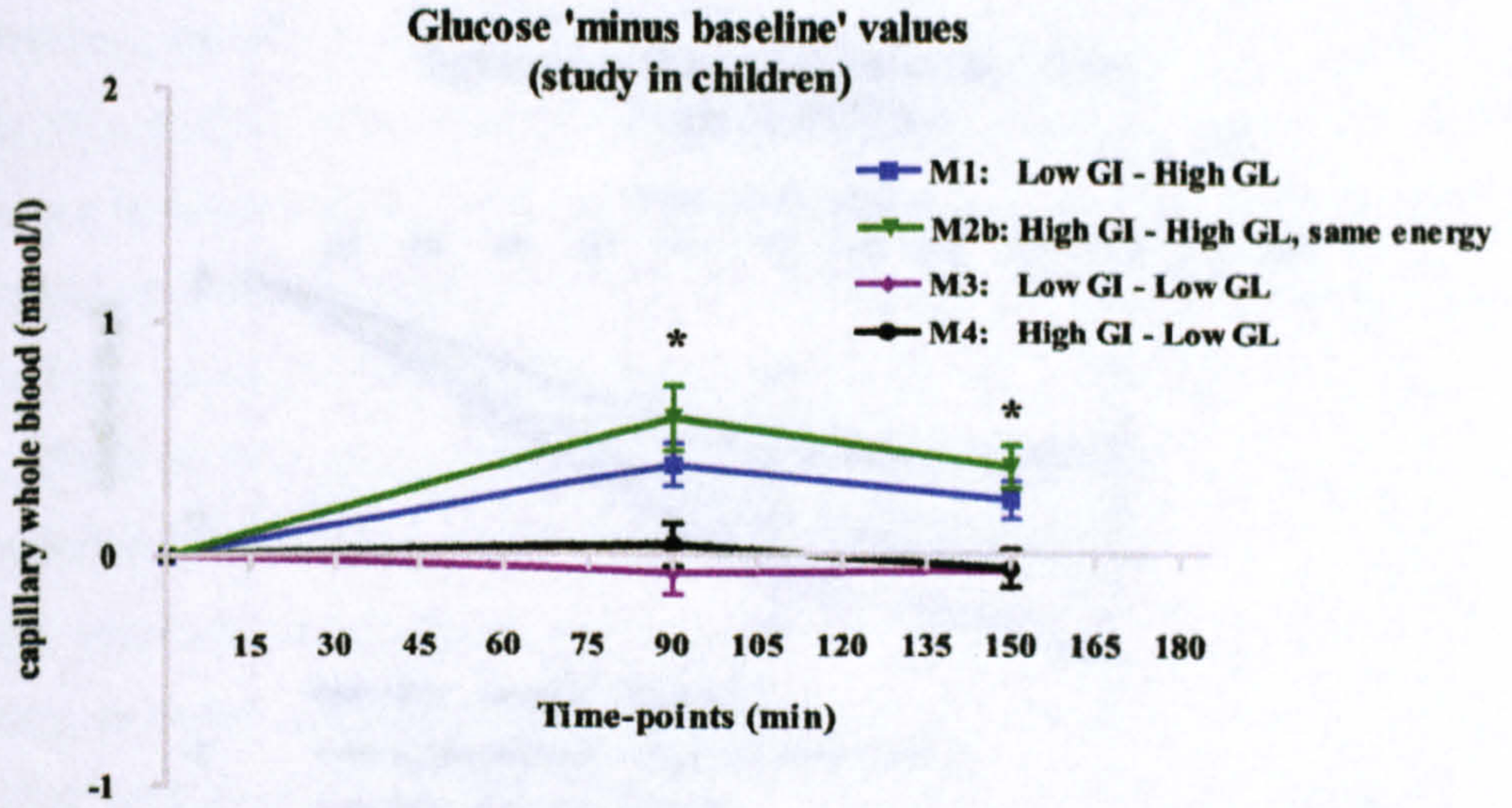
* As measured on the non-testing day/ † Un-paired t-test; ‡ Time finger pick (fp) blood sample was taken to measure glucose; Two-tailed significance, $p<0.05$

Table 4.17: Blood glucose and salivary cortisol levels, and time these were taken, in 74 children participating in the study, all children, and in the four GI and GL groups.

	All children		High GL				Low GL							
			Low GI (M1)		High GI (M2)		Low GI (M3)		High GI (M4)					
n (females/males)	74		35 (17/18)		35 (17/18)		39 (20/19)		39 (20/19)					
	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	p‡	GI	p** GLxGI	GL
Time of breakfast (AM)	08:46:31	00:00:45	08:46:20	00:01:57	08:48:00	00:01:32	08:44:33	00:01:02	08:47:18	00:01:28	0.400			
Time finger prick baseline (AM)	08:32:58	00:00:38	08:32:59	00:01:17	08:33:29	00:01:26	08:31:27	00:00:56	08:34:00	00:01:24	0.510			
BG baseline (mmol/L)	5.1	0.0	5.1	0.1	5.1	0.1	5.1	0.1	4.9	0.1		0.131	***0.032***	0.359
Time finger prick 'before' (AM)	10:18:48	00:00:42	10:19:57	00:02:07	10:19:37	00:01:19	10:17:36	00:01:05	10:18:14	00:00:42	0.614			
BG 'before' (mmol/L)	5.3	0.1	5.5	0.1	5.6	0.1	5.0	0.1	5.0	0.1		0.245	0.275	***<0.001***
Time finger prick 'after' (AM)	11:13:02	00:01:14	11:12:11	00:03:13	11:13:45	00:02:41	11:10:50	00:01:47	11:15:19	00:02:15	0.599			
BG 'after' (mmol/L)	5.1	0.0	5.3	0.1	5.4	0.1	5.0	0.1	4.9	0.1		0.960	0.111	***0.001***
BG 'before-baseline' (mmol/L)	0.2	0.1	0.4	0.1	0.6	0.1	-0.1	0.1	0.0	0.1		0.054	0.582	***<0.001***
BG 'after-baseline' (mmol/L)	0.1	0.0	0.2	0.1	0.4	0.1	-0.1	0.1	-0.1	0.1		0.251	0.353	***<0.001***
BG 'after-before' (mmol/L)	-0.1	0.1	-0.1	0.1	-0.2	0.2	0.0	0.1	-0.1	0.1		0.403	0.878	0.238
Time cortisol baseline* (AM)	08:32:26	00:01:24												
Cortisol baseline* (ng/mL)	5.1	0.2												
Time cortisol baseline (AM)	08:25:57	00:00:36	08:25:02	00:01:06	08:26:28	00:01:29	08:24:57	00:00:56	08:27:21	00:01:14	0.416			
Cortisol baseline (ng/mL)	4.8	0.1	5.1	0.3	4.9	0.3	4.8	0.3	4.4	0.2		***0.039***	0.653	0.237
Time cortisol before (AM)	10:16:20	00:00:51	10:16:31	00:02:05	10:16:55	00:02:04	10:14:35	00:01:03	10:17:23	00:01:32	0.654			
Cortisol before (ng/mL)	3.7	0.1	3.9	0.2	3.9	0.2	3.5	0.2	3.6	0.2		0.753	0.280	0.142
Time cortisol 'after' (AM)	11:09:25	00:01:11	11:08:56	00:03:03	11:09:32	00:02:36	11:07:07	00:01:39	11:12:01	00:02:09	0.518			
Cortisol 'after' (ng/mL)	3.6	0.1	3.7	0.2	3.8	0.2	3.3	0.1	3.6	0.1		0.057	0.266	0.267
Cortisol 'before-baseline' (ng/mL)	-1.1	0.1	-1.2	0.2	-1.1	0.2	-1.3	0.2	-0.8	0.2		***0.032***	0.271	0.696
Cortisol 'after-baseline' (ng/mL)	-1.2	0.1	-1.6	0.3	-1.2	0.2	-1.5	0.3	-0.8	0.2		***0.003***	0.353	0.397
Cortisol 'after-before' (ng/mL)	-0.1	0.1	-0.3	0.2	-0.1	0.2	-0.1	0.1	0.0	0.1		0.237	0.880	0.532

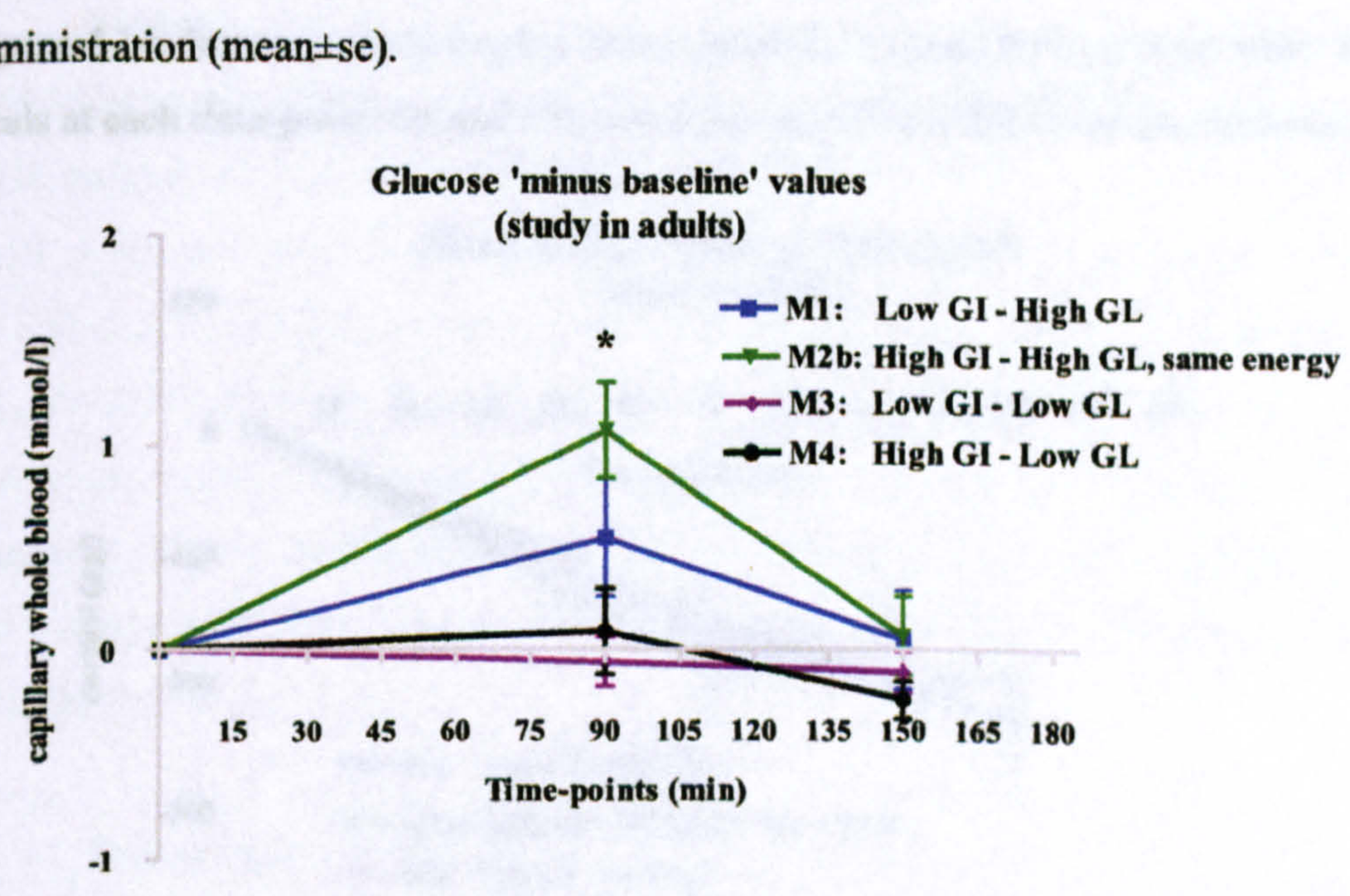
* As measured on the non-testing day/ † Un-paired t-test; ‡ One-way ANOVA; ** Repeated measures ANOVA (GI within-subject factor, GL between-subject factor); Two-tailed significance, ***p<0.05***, 0.05≤p≤0.08

Figure 4.11: Capillary whole blood glucose levels ('minus baseline' values) for each one of the four meals at each time-point: before and after the CF tests (mean±se).



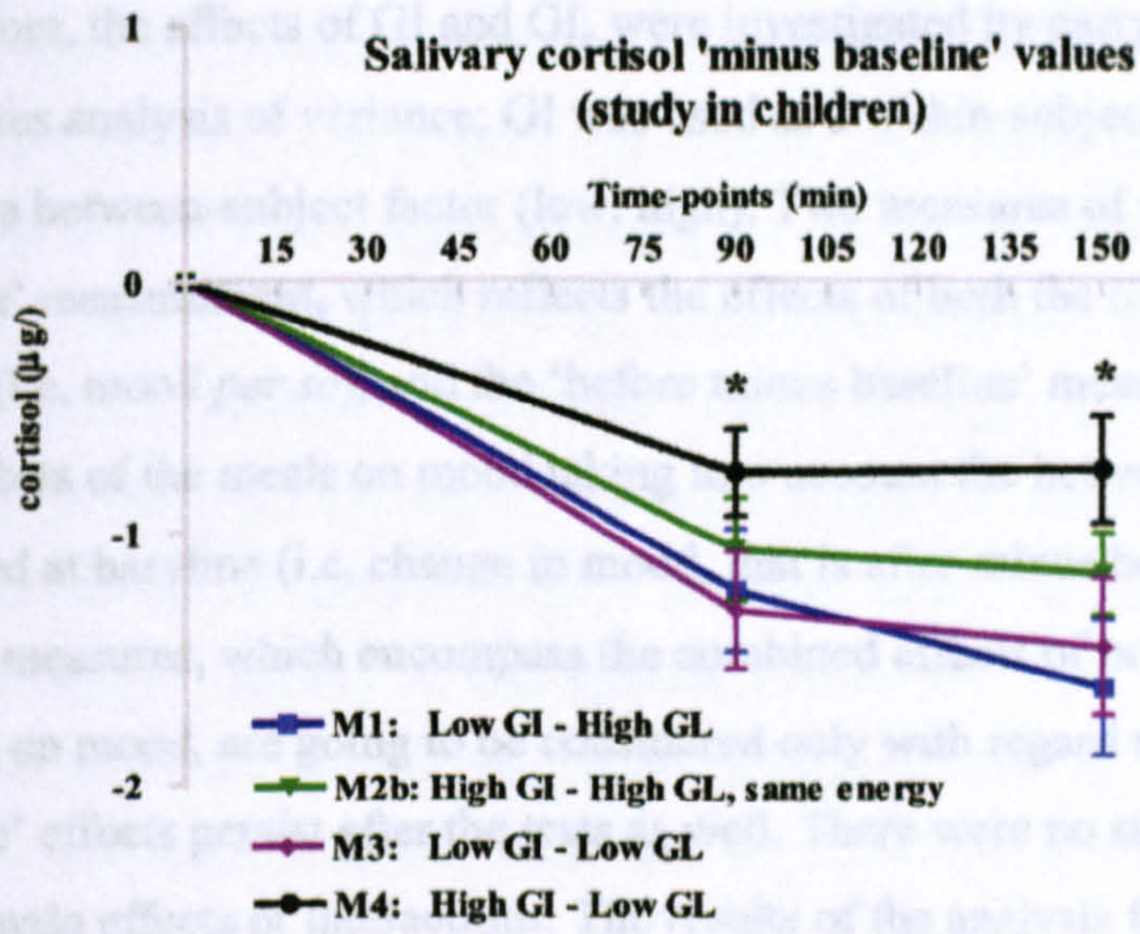
*Statistically significant GL differences; see section 4.3.2.a, page 173 for details.

Figure 4.12: Capillary whole blood glucose levels ('minus baseline' values) for each one of the four meals at each time-point: 90 and 150 min after breakfast administration (mean±se).



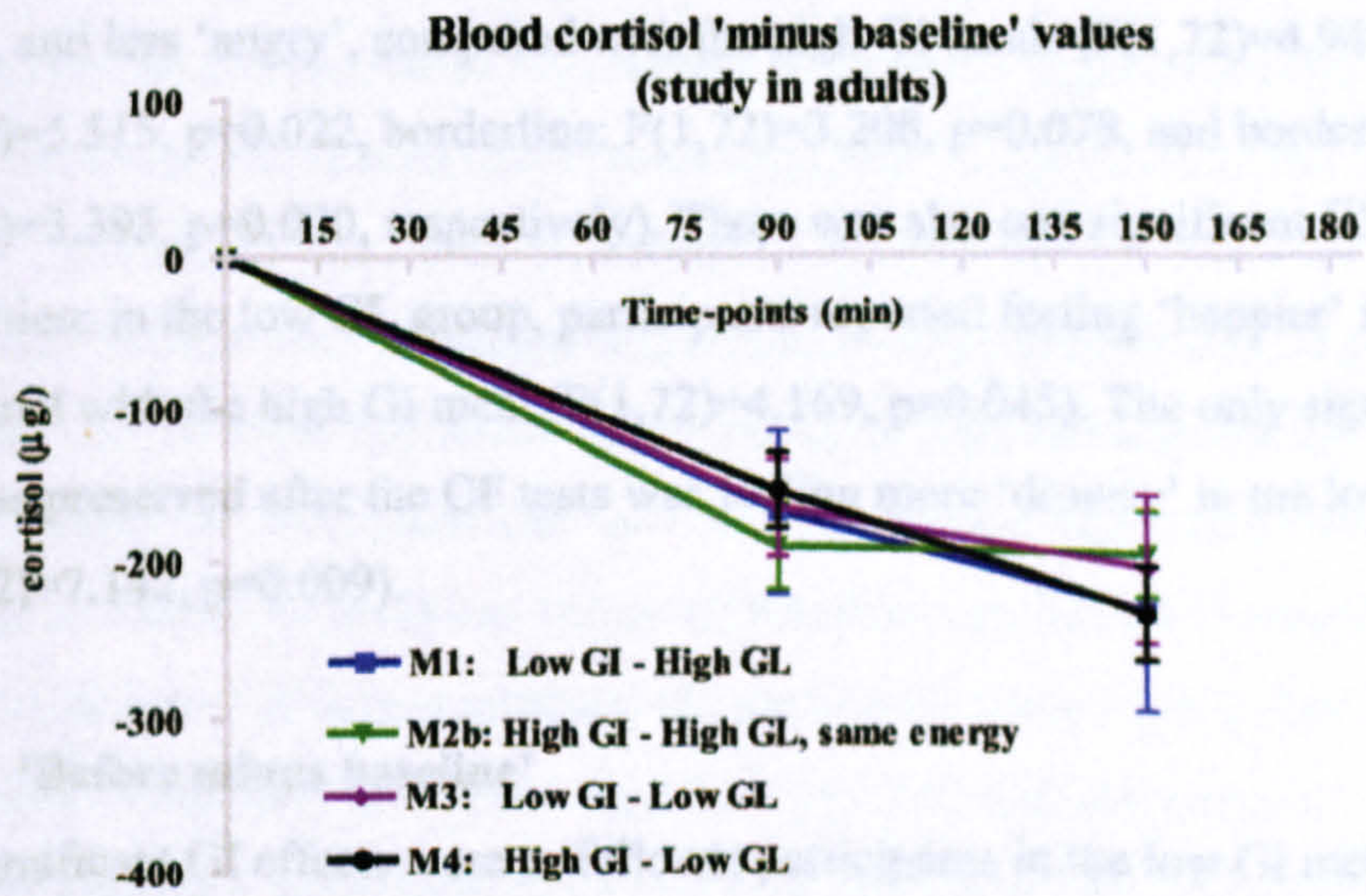
*Statistically significant GL differences; see section 4.2.2.a, page 144 for details.

Figure 4.13: Salivary cortisol levels ('minus baseline' values) for each one of the four meals at each time-point: before and after the CF tests (mean±se).



*Statistically significant GI differences; see section 4.3.2.b, page 174 for details.

Figure 4.14: Serum cortisol levels ('minus baseline' values) for each one of the four meals at each time-point: 90 and 150 min after breakfast administration (mean±se).



4.3.3 Mood and breakfast meals

Another important factor that could be affected by meal administration is mood. Therefore, the effects of GI and GL were investigated by carrying out a repeated measures analysis of variance; GI was used as a within-subject factor (low, high), and GL as a between-subject factor (low, high). Two measures of mood were used: the 'before' measurement, which reflects the effects of both the meals and the baseline mood (i.e. mood *per se*); and the 'before minus baseline' measurement, which reflects the effects of the meals on mood taking into account the between-subject differences in mood at baseline (i.e. change in mood, that is after minus before the meal). The 'after' measures, which encompass the combined effects of both the meals and the CF testing on mood, are going to be considered only with regard to whether the observed 'before' effects persist after the tests as well. There were no significant gender or order main effects or interactions. The results of the analysis for the 'before' and the 'before minus baseline' mood scales are presented in Table 4.18 and Table 4.19, respectively.

❖ **'Before'**

With regard to the 'before' mood scales, there were a few significant GI effects. Participants in the low GI meals reported feeling more 'drowsy', more 'sad', more 'tired', and less 'angry', compared with the high GI meals ($F(1,72)=4.942$, $p=0.029$, $F(1,72)=5.515$, $p=0.022$, borderline: $F(1,72)=3.208$, $p=0.078$, and borderline: $F(1,72)=3.393$, $p=0.070$, respectively). There was also one significant GL*GI interaction: in the low GL group, participants reported feeling 'happier' in the low GI compared with the high GI meal ($F(1,72)=4.169$, $p=0.045$). The only significant effect that was preserved after the CF tests was feeling more 'drowsy' in the low GI meals ($F(1,72)=7.142$, $p=0.009$).

❖ **'Before minus baseline'**

The significant GI effects were as follows: participants in the low GI meals reported feeling less 'nervous', 'happier', 'sadder', more 'alert', and less 'thirsty' compared with the high GI meals ($F(1,72)= 4.019$, $p=0.049$, $F(1,72)=4.265$, $p=0.043$, borderline: $F(1,72)=3.658$, $p=0.060$, borderline: $F(1,72)=4.217$, $p=0.060$, and borderline: $F(1,72)=3.755$, $p=0.057$, respectively). There were also three significant GI*GL

interactions: in the low GL group participants reported feeling 'calmer', more 'dissatisfied', and less 'contented' in the low GI than in the high GI meal, while in the high GL group the same applied for the high GI rather than the low GI meal (borderline: F(1,72)=3.610, p=0.061, F(1,72)=5.473, p=0.022, and borderline: F(1,72)=3.393, p=0.070, respectively). Finally, there were observed four significant GL effects: participants reported feeling less 'confident', and more 'sluggish', 'hungry', and 'thirsty' in the low GL meals, compared with the high GL meals (F(1,72)=6.998, p=0.003, F(1,72)=6.816, p=0.011, F(1,72)=7.414, p=0.008, and F(1,72)=4.886, p=0.030, respectively). The effects that were preserved after the CF tests include the GI effect on feeling 'nervous', 'alert' and 'thirsty', the GL effect on feeling 'hungry' and the GI*GL interaction on feeling 'calm' (F(1,72)=6.419, p=0.013, borderline: F(1,72)=3.855, p=0.053, borderline: F(1,72)=3.493, p=0.066, F(1,72)=4.577, p=0.036, and borderline: F(1,72)=3.473, p=0.066, respectively).

When the 'before' measurement was assessed there were observed only two significant GI associations; the low GI meals appeared to be associated with feeling more 'drowsy' and 'sad'. There were also two borderline significant associations; in the low GI meals children reported feeling more 'tired' and less 'angry'.

The 'before minus baseline' analysis yielded both significant GI and GL effects. In the high GL groups the results were highly statistically significant and consistent, as participants reported feeling more 'confident', less 'sluggish', less 'hungry' and less 'thirsty', that is they had improved mood immediately before the CF tests. In relation to GI, the findings were borderline significant with the exception of 'happy' and 'nervous' which were significant; in the low GI groups participants reported feeling more 'happy' and 'alert', and less 'nervous' and 'thirsty' (i.e. improved mood). The findings in relation to breakfast and mood whether significant or borderline were in the same direction with one exception; in the low GI groups pupils reported feeling more 'sad'. Therefore, the GI and the GL of the breakfast had an effect on the mood state participants were before the CF tests; the low GI and the high GL groups were associated with improved mood.

From the analysis for the 'before' and the 'before minus baseline' mood scales it can be seen that the 'before minus baseline' measure yielded more significant results, which in fact represent the net effect of the meals on mood. Thus, the rate of change in mood after the meal administration rather than mood *per se* 'before' would be expected to have an effect on CF test performance, if any.

Therefore, the next step was to enter in the repeated measures ANOVA variables (i.e. confounders) that could affect the outcome (i.e. factors and covariates), and to investigate whether these other variables explain the variance of the 'before minus baseline' mood states (22 in total). These factors and covariates were entered in the model as blocks (i.e. four in total):

1. Order of administration of the different types of breakfast as a main factor - to investigate whether the order of the visit has an effect on cognitive performance
2. Gender as a main factor - to investigate whether there are differences between males and females
3. Age, height, weight and BMI as covariates - these variables can be considered as a proxy of physiological maturity
4. Glucose and cortisol values. These would be explanatory variables in the pathway between breakfast and cognitive function, and were also entered in the analysis as covariates. Hence, the final block of covariates included the glucose and cortisol values before the tests; the analysis was repeated first for the 'before minus baseline' measure, which takes into account the baseline variations, and then for the 'before' measure to investigate whether the rate of change in these measures or their values *per se* are associated with performance.

Social-class, the habitual breakfast eating habits of the participants, the number of days between the two testing visits, as well as Hb levels were not associated with mood. Therefore, the repeated measures analysis was carried out for each one of the mood states, adding one by one the four blocks above in order to reveal the best prediction models.

This analysis revealed that there were no significant effects of gender, order, age, height, weight, BMI, blood glucose and salivary cortisol values (either rate of change or values *per se*) on the mood states. Therefore, the observed significant GI and GL effects can be attributed to the meal ingested, and were not confounded by the factors listed above.

Table 4.18: Comparison of the Mood Scales before the CF tests among the four GI and GL groups.

MOOD SCALES 'BEFORE'	HIGH GL				LOW GL						
	LOW GI		HIGH GI		LOW GI		HIGH GI		p*		
	Mean	se	Mean	se	Mean	se	Mean	se	GI	GI*GL	GL
Friendly	3.1	0.1	3.0	0.1	3.1	0.1	3.1	0.1	0.436	0.845	0.724
Nervous	0.4	0.1	0.5	0.2	0.5	0.2	0.5	0.1	0.400	0.533	0.811
Drowsy	0.6	0.2	0.5	0.1	0.6	0.1	0.3	0.1	***0.029***	0.312	0.632
Happy	2.9	0.1	2.9	0.1	3.1	0.1	2.9	0.2	0.199	***0.045***	0.537
Calm	2.9	0.1	2.8	0.2	2.9	0.1	2.7	0.2	0.133	0.489	0.919
Uncertain	0.3	0.1	0.4	0.1	0.3	0.1	0.4	0.1	0.211	0.752	0.729
Sad	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.0	***0.022***	0.506	0.975
Energetic	2.5	0.2	2.5	0.2	2.7	0.2	2.4	0.2	0.197	0.197	0.547
Muddled	0.2	0.1	0.1	0.1	0.4	0.1	0.4	0.1	0.851	0.621	0.122
Relaxed	2.7	0.2	2.7	0.1	2.7	0.2	2.8	0.2	0.866	0.553	0.733
Dissatisfied	0.1	0.0	0.1	0.1	0.2	0.1	0.2	0.1	0.729	0.363	0.447
Alert	2.5	0.2	2.3	0.2	2.6	0.2	2.5	0.2	0.401	0.890	0.689
Confident	2.9	0.2	2.9	0.2	2.9	0.2	2.8	0.1	0.925	0.925	0.992
Tired	1.1	0.2	0.9	0.2	0.9	0.2	0.7	0.2	0.078	0.982	0.323
Angry	0.0	0.0	0.1	0.1	0.1	0.1	0.2	0.1	0.070	0.720	0.123
Contented	2.1	0.2	2.0	0.2	2.1	0.2	2.2	0.2	0.820	0.673	0.723
Lively	2.5	0.2	2.7	0.2	2.6	0.2	2.6	0.2	0.776	0.345	0.959
Tense	0.4	0.1	0.5	0.2	0.6	0.1	0.6	0.1	0.638	0.799	0.262
Sluggish	0.3	0.1	0.3	0.1	0.5	0.1	0.4	0.1	0.788	0.345	0.306
Clearheaded	2.1	0.2	2.1	0.2	2.5	0.2	2.5	0.2	0.726	0.726	0.115
Hungry	0.8	0.2	0.9	0.2	0.8	0.2	0.9	0.2	0.531	0.858	0.861
Thirsty	1.1	0.2	1.3	0.2	0.7	0.2	0.9	0.2	0.085	0.845	0.091

* Repeated measures ANOVA (GI within-subject factor, GL between-subject factor).

Two-tailed significance, ***p<0.05***, 0.05≤p<0.08.

Table 4.19: Comparison of the Mood Scales 'before minus baseline' among the four GI and GL groups.

MOOD SCALES 'BEFORE-BASELINE'	HIGH GL				LOW GL						
	LOW GI		HIGH GI		LOW GI		HIGH GI		p*		
	Mean	se	Mean	se	Mean	se	Mean	se	GI	GI*GL	GL
Friendly	0.5	0.1	0.3	0.1	0.5	0.1	0.5	0.1	0.587	0.435	0.478
Nervous	-0.6	0.2	-0.2	0.2	-0.4	0.2	-0.1	0.1	***0.049***	0.787	0.347
Drowsy	-0.6	0.2	-0.4	0.1	-0.4	0.2	-0.3	0.1	0.344	0.891	0.366
Happy	0.5	0.1	0.2	0.1	0.3	0.1	0.1	0.1	***0.043***	0.998	0.283
Calm	0.0	0.1	0.2	0.2	0.3	0.1	0.0	0.1	0.747	0.061	0.680
Uncertain	-0.1	0.1	-0.1	0.1	-0.3	0.1	-0.3	0.1	0.760	0.907	0.221
Sad	0.1	0.1	-0.1	0.1	0.1	0.1	-0.1	0.1	0.060	0.965	0.526
Energetic	0.9	0.2	0.4	0.2	0.5	0.2	0.4	0.2	0.092	0.211	0.408
Muddled	-0.1	0.1	-0.1	0.1	-0.1	0.1	0.0	0.1	0.758	0.423	0.662
Relaxed	0.4	0.2	0.3	0.2	0.1	0.1	0.2	0.1	0.972	0.345	0.233
Dissatisfied	-0.1	0.1	0.0	0.0	0.1	0.1	-0.1	0.1	0.931	***0.022***	0.337
Alert	0.5	0.2	0.2	0.2	0.6	0.2	0.3	0.2	0.060	0.979	0.633
Confident	0.4	0.2	0.3	0.1	0.1	0.1	-0.2	0.1	0.267	0.707	***0.003***
Tired	-0.6	0.2	-0.7	0.1	-0.6	0.2	-0.6	0.2	0.609	0.898	0.913
Angry	-0.1	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.217	0.355	0.488
Contented	0.1	0.2	-0.1	0.2	-0.3	0.2	0.2	0.2	0.469	0.070	0.749
Lively	0.6	0.2	0.5	0.2	0.5	0.2	0.2	0.1	0.240	0.690	0.271
Tense	-0.1	0.1	-0.1	0.2	-0.2	0.2	-0.1	0.1	0.858	0.858	0.645
Sluggish	-0.6	0.2	-0.3	0.1	-0.1	0.1	-0.1	0.1	0.258	0.354	***0.011***
Clearheaded	0.3	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.673	0.561	0.997
Hungry	-2.1	0.2	-2.0	0.2	-1.5	0.2	-1.2	0.2	0.124	0.423	***0.008***
Thirsty	-1.6	0.2	-1.1	0.2	-0.9	0.2	-0.8	0.2	0.057	0.375	***0.030***

* Repeated measures ANOVA (GI within-subject factor, GL between-subject factor).

Two-tailed significance, ***p<0.05***, 0.05≤p<0.08.

4.3.4 Cognitive Function and breakfast meals

The same CF tests were used as in the cross-sectional study. Similar to the cross-sectional study, in spite of some apparent disparities from normality, parametric tests were used to explore the CF test scores. The mean time that the CF testing started (i.e. start time of the first CF test) was 102.9 min (se=0.3) after breakfast and finished (i.e. finish time of the last CF test) 136.1 min (se=0.6) after breakfast; this effectively means that the entire CF testing lasted 33.2 min (se=0.4). There were no differences in the time that the CF testing started or how long it lasted between the four GI and GL groups (one-way ANOVA, p=0.780, p=0.996, respectively). The average number of days between the first and the second visit was 14.7 (se=0.2).

The difficulty assigned to each one of the CF tests by the participants was the same between their first and their second visit. The most difficult task was delayed recall, followed by serial sevens, immediate word recall speed of information processing, Stroop task, word generation and matrices (Friedman test, $p<0.001$). Nonetheless, the difficulty assigned to each one of the CF tests was not the same for the low or the high GI (Friedman test, $p<0.001$). Therefore, in the present study task difficulty cannot be associated with performance, as it is not consistent between the two GI meals. Besides, difficulty is a consequence of the test procedure rather than a factor that would influence the outcome (like the type of breakfast or mood). Paired t-tests revealed that there were no differences between the two visits with regard to the difficulty assigned to each test, while there were differences between the two GI meals (low, high).

Correlation analysis was carried out between BG and CF/ Mood ('before minus baseline') and cortisol and CF/ Mood ('before minus baseline'), to reveal potential associations between pre-task levels (i.e. baseline, 'before' and 'before minus baseline'), change in levels during the testing ('after minus before'), as well as the post-task levels ('after', 'after minus baseline'). Having looked at these associations with glucose only in the cross-sectional study, it was observed that there was not anything there. In the present study, similarly, there were no consistent findings relating to either GI or GL. Besides, the present study was not set out to investigate whether differences in glucose or cortisol levels are associated with differences in

cognitive function or mood. Similarly, there were not observed any consistent patterns when mood was correlated with CF.

Repeated measures ANOVA was carried out initially to compare the means among the four groups of subjects with regard to their performance on the CF tests, using only GI and GL as factors; GI was used as a within-subject factor (low, high), and GL as a between-subject factor (low, high). This was done to explore whether GI and GL have an effect on cognition, ignoring at first any effects of potential confounders. The results for all possible CF test outcomes are presented in Table 4.20. This analysis resulted in only a few significant GI associations. Stroop task performance was better in the high GI meal, but only in the high GL group (borderline: $F(1,72)=3.396$, $p=0.069$). Furthermore, speed of information processing task performance was better in the high GI meals compared with the low GI meals, as assessed by both 'correct', and 'correct minus incorrect' responses ($F(1,72)=4.701$, $p=0.033$, and $F(1,72)=4.504$, $p=0.037$, respectively). The remaining main and non-main CF test variables were unrelated to GI, GL or GI*GL interaction.

Table 4.20: Comparison of cognitive function test scores* among the four GI and GL groups.

	HIGH GL				LOW GL						
	Low GI		High GI		Low GI		High GI		p†		
	Mean	se	Mean	se	Mean	se	Mean	se	GI	GI*GL	GL
Word generation task 'correct'	15.0	0.8	15.4	0.8	15.2	0.7	14.4	0.8	0.761	0.360	0.664
• 'errors'	0.1	0.1	0.3	0.1	0.2	0.1	0.3	0.1	0.108	0.723	0.995
• 'correct-errors'	14.9	0.8	15.1	0.8	15.0	0.8	14.1	0.8	0.585	0.398	0.666
Word recall immediate 'correct'	6.7	0.4	6.6	0.3	7.2	0.3	7.3	0.3	0.930	0.554	0.143
• 'errors'	0.6	0.2	0.6	0.2	0.5	0.1	0.5	0.1	0.905	0.905	0.558
• 'correct-errors'	6.1	0.4	6.0	0.4	6.7	0.4	6.8	0.3	0.979	0.643	0.158
Stroop task 'control' time	47.3	2.0	44.9	1.7	47.2	1.5	47.5	1.6	0.186	0.069	0.575
• 'actual' time	75.8	3.1	74.6	2.8	77.8	2.0	77.7	2.3	0.579	0.644	0.457
• 'interference'‡ score	28.6	2.0	29.7	2.2	30.7	1.7	30.2	1.8	0.803	0.527	0.596
• 'correct'	58.7	0.4	58.9	0.3	59.2	0.2	59.1	0.3	0.919	0.401	0.294
• 'errors'	1.3	0.4	1.1	0.3	0.8	0.2	0.9	0.3	0.917	0.389	0.290
• 'correct-errors'	57.4	0.7	57.8	0.6	58.5	0.4	58.1	0.5	0.918	0.393	0.291
Matrices 'correct'	11.8	0.4	12.2	0.4	12.3	0.4	12.0	0.4	0.768	0.180	0.756
Speed of information processing 'correct'	12.7	0.7	13.0	0.7	12.4	0.7	13.9	0.7	*0.033*	0.197	0.717
• 'accuracy' score	0.8	0.0	0.8	0.0	0.8	0.0	0.8	0.0	0.344	0.796	0.548
• 'misses'	4.8	2.4	3.8	1.6	3.6	0.7	3.1	0.5	0.234	0.732	0.608
• 'errors'	1.5	0.9	1.3	0.9	0.7	0.2	0.8	0.3	0.763	0.499	0.466
• 'correct-errors'	11.1	1.3	11.7	1.4	11.7	0.7	13.1	0.8	*0.037*	0.372	0.504
Serial sevens 'correct'	16.3	1.8	17.2	1.7	15.1	1.9	15.5	1.8	0.298	0.710	0.558
• 'errors'	3.8	0.4	4.0	0.6	4.0	0.5	4.8	0.7	0.284	0.574	0.456
• 'correct-errors'	12.5	2.0	13.2	2.0	11.1	2.2	10.7	1.7	0.855	0.581	0.456
Word recall delayed 'correct'	4.5	0.4	4.8	0.3	5.1	0.4	5.4	0.4	0.299	0.878	0.186
• 'errors'	1.0	0.2	0.9	0.2	0.7	0.1	0.8	0.2	0.891	0.484	0.433
• 'correct-errors'	3.5	0.5	3.9	0.5	4.4	0.4	4.6	0.5	0.350	0.855	0.175
• 'forgetting'**	2.1	0.3	1.8	0.2	2.0	0.3	1.9	0.2	0.236	0.650	0.938

* All possible CF test scores' variables are presented; † Repeated measures ANOVA (GI within-subject factor, GL between-subject factor); ‡ Stroop task 'actual' minus 'control' time;

**Word recall immediate 'correct' minus word recall delayed 'correct'; Two-tailed significance, $p<0.05$, $0.05\leq p<0.08$.

The next stage in the analysis was to enter in the repeated measures ANOVA those variables that could affect the outcome (i.e. factors and covariates), and to investigate whether these other variables explain the variance of the main CF score variables (nine in total). These factors and covariates were entered in the model as blocks (i.e. five in total):

1. Order as a main factor - to investigate whether the order of the visit has an effect on cognitive performance
2. Gender as a main factor - to investigate whether there are differences between males and females
3. Age, height, weight and BMI as covariates - these variables can be considered as a proxy of physiological maturity
4. The mood states that were associated with the meals administered as covariates. These mood states included the 'before minus baseline' measure and only the ones that were consistent and significantly associated with GI and GL ($p<0.05$) and not with the GI*GL interaction (the latter would complicate the interpretation of the findings). Therefore, 'nervous', 'happy' (i.e. GI effect), and 'confident', 'sluggish', 'hungry' and 'thirsty' (i.e. GL effect) were entered as covariates. Mood *per se* before the CF tests did not reflect the net potential effect of the meals, and as such was not entered in the analysis as a covariate (when it was entered it did not explain any of the variance).
5. Glucose and cortisol values would be explanatory variables in the pathway between breakfast and cognitive function, and were also entered in the analysis as covariates. Hence, the final block of covariates included the glucose and cortisol values before the tests; the analysis was repeated first for the 'before minus baseline' measure, which takes into account the baseline variations, and then for the 'before' measure to investigate whether the rate of change in these measures or their values *per se* are associated with performance.

Social-class, the habitual breakfast eating habits of the participants, the number of days between the two testing visits, as well as Hb levels were not associated with performance. Therefore, the repeated measures analysis was carried out for each one of the main CF test outcomes, adding one by one the previously mentioned five blocks in order to reveal the best prediction models.

Word generation task ('correct')

The addition of visit explained some of the variance as a within-subject variable; participants performed better on their second visit compared with their first. The overall GI, GL effects or GI*GL interaction remained non-significant. Therefore, visit was kept in the model. Gender was unrelated to performance, and subsequently removed from the model. When age, height, weight, and BMI were entered in the analysis with order, it appeared that age *per se* was not as important as the remaining variables; the latter explained some of the variance as between-subject factors (i.e. a better indication of physiological maturity), though the overall GI, GL effect or GI*GL interaction remained non-significant. These variables also overrode the visit effect. Therefore, visit and age were removed from the model. When height, weight, and BMI were taken into account, the GI effect became significant ($F(1,69)=4.218$, $p=0.044$); students in the low GI groups performed better than students in the high GI groups. Then, mood was entered in the analysis with the indicators of physiological maturity (i.e. height, weight, BMI). The addition of mood overrode the height, weight, and BMI effect (i.e. no longer significant). These covariates were removed from the model, and the GI effect became more significant ($F(1,60)=5.248$, $p=0.026$).

Following that, the 'before minus baseline' cortisol and glucose levels were entered in the model with mood; the addition of these variables decreased significance suggesting that mood and these values are correlated ($F(1,54)=3.387$, $p=0.071$). It could be argued that these are just explanatory variables in the pathway between breakfast, mood and cognitive function. Similarly, the before cortisol and glucose levels were entered in the model with mood; the addition of these variables removed significance, and it could be argued that the change in glucose and cortisol levels rather than the values *per se* explain part of the variance.

Immediate word recall ('correct')

Visit did not explain any of the variance with regard to the performance on this task. The overall GI, GL effect or GI*GL interaction remained non-significant. Therefore, visit was removed from the model. Similarly, gender was unrelated to performance, and as such removed from the model. When age, height, weight, and BMI were entered in the analysis, it appeared that age *per se* was not as important as the remaining variables. Height, weight, and BMI explained some of the variance as between-subject factors, though the overall GI, GL effect or GI*GL interaction

remained non-significant. Therefore, age was removed from the model. Then, mood was entered in the analysis with height, weight, and BMI. The addition of mood did not explain the variance and the overall p-value of the model was not significant. Height, weight, and BMI remained in the model as significant between-subject factors. Following that, the 'before minus baseline' cortisol and glucose levels were entered in the model with height, weight, and BMI; the addition of these variables did not explain the variance, while height, weight, and BMI remained in the model as significant between-subject factors. Similarly, the 'before' cortisol and glucose levels were entered in the model with height, weight, and BMI; similar findings were observed as for the 'before minus baseline' values. Therefore, for this task, height, weight, and BMI helped explain part of the between-subject variance ($F(1,69)=5.502$, $p=0.022$, $F(1,69)=5.480$, $p=0.022$, and $F(1,69)=5.723$, $p=0.019$, respectively), without affecting the overall GI, GL effect or GI*GL interaction.

Stroop task ('control', 'actual', 'interference' score)

<u>Control task:</u>

The addition of visit explained some of the variance as a within-subject variable; participants performed better on their second visit compared with their first. The overall GI and GL effects remained non-significant, while the GI*GL interaction was borderline significant ($F(1,71)=3.432$, $p=0.068$). Therefore, visit was kept in the model. Gender was unrelated to performance, and as such removed from the model. When age, height, weight, and BMI were entered in the analysis with visit, these were not significantly associated with performance, and in addition removed the borderline significant GI*GL interaction. Consequently, age, height, weight and BMI were removed from the model. Then, mood was entered in the analysis with visit, which did not help explain the variance, and furthermore removed significant GI*GL interaction. Therefore, mood was removed from the analysis. Visit and the 'before minus baseline' glucose and cortisol levels were subsequently entered in the analysis, which did not explain any of the variance. Finally, the glucose and cortisol values *per se* were entered in the analysis. The addition of these variables overrode the visit effect (which was therefore removed from the model), and furthermore resulted in a significant GI*GL interaction ($F(1,67)=4.912$, $p=0.030$); that is in the high GL group only, participants in the high GI meal performed better than participants in the low GI meal.

Actual task:

The addition of visit explained some of the variance as a within-subject variable; participants performed better on their second visit compared with their first. The overall GI, GL effect or GI*GL interaction remained non-significant. Therefore, visit remained in the model. Gender was unrelated to performance, and as such removed from the model. When age, height, weight, and BMI were entered in the analysis with visit, these were not significantly associated with performance, and the overall GI, GL effect or GI*GL interaction remained non-significant. Consequently, age, height, weight and BMI were removed from the model. Then, mood was entered in the analysis with visit, which did not help explain the variance, and the overall GI, GL effect and GI*GL interaction remained non-significant. Therefore, mood was removed from the analysis. Visit and the 'before minus baseline' glucose and cortisol levels were subsequently entered in the analysis, which did not explain any of the variance, and the overall GI, GL effect and GI*GL interaction remained non-significant. Finally, the glucose and cortisol values *per se* were entered in the analysis. The addition of these variables similarly did not explain any of the variance, and the overall GI, GL effect and GI*GL interaction remained non-significant. Therefore, for this task, only visit helped explain some of the within-subject variance ($F(1,71)=27.704$, $p<0.001$), without affecting the overall GI, GL effect or GI*GL interaction.

'Interference' score:

The addition of visit explained some of the variance as a within-subject variable; participants performed better on their second visit compared with their first visit. The overall GI, GL effect or GI*GL interaction remained non-significant. Therefore, visit remained in the model. Gender was a significant between-subject factor, suggesting that boys performed better than girls on this task; as such it remained in the model. When age, height, weight, and BMI were entered in the analysis with visit and gender, these were not significantly associated with performance, and the overall GI, GL effect or GI*GL interaction remained non-significant. Consequently, age, height, weight and BMI were removed from the model. Then, mood was entered in the analysis with visit and gender, which did not help explain the variance, and the overall GI, GL effect and GI*GL interaction remained non-significant. Therefore, mood was removed from the analysis. Visit and gender and the 'before minus baseline' glucose

and cortisol levels were subsequently entered in the analysis, which did not explain any of the variance, and the overall GI, GL effect and GI*GL interaction remained non-significant. Finally, the glucose and cortisol values *per se* were entered in the analysis. The addition of these variables similarly did not explain any of the variance, and the overall GI, GL effect and GI*GL interaction remained non-significant. Therefore, for this task, visit helped explain some of the within-subject variance ($F(1,70)=8.337$, $p=0.005$), and gender some of the between-subject variance ($F(1,70)=5.647$, $p=0.020$), without affecting the overall GI, GL effect or GI*GL interaction.

Matrices ('correct')

The addition of visit did not explain any of the variance as a within-subject variable, and the overall GI, GL effect or GI*GL interaction remained non-significant. Therefore, visit was removed from the model. Similarly, gender was unrelated to performance, and subsequently removed from the model. When age, height, weight, and BMI were entered in the analysis, only height explained some of the between-subject variance, and the overall GI, GL effect or GI*GL interaction remained non-significant. Consequently, age, weight and BMI were removed from the model. Then, mood was entered in the analysis with height, which did not help explain the variance, and the overall GI, GL effect and GI*GL interaction remained non-significant. Therefore, mood was removed from the analysis. Height and the 'before minus baseline' glucose and cortisol levels were subsequently entered in the analysis, which did not explain any of the variance, and the overall GI, GL effect and GI*GL interaction remained non-significant. Finally, the glucose and cortisol values *per se* were entered in the analysis. The addition of these variables similarly did not explain any of the variance, and the overall GI, GL effect and GI*GL interaction remained non-significant. Therefore, for this task, height helped explain part of the between-subject variance ($F(1,71)=11.171$, $p=0.001$), without affecting the overall GI, GL effect or GI*GL interaction.

Speed of information processing ('correct')

The addition of visit explained some of the variance as a within-subject variable; participants performed better on their second visit compared with their first. The overall GL effect and GI*GL interaction remained non-significant, while the GI effect became significant ($F(1,71)=4.921$, $p=0.030$). This effectively means that participants performed better in the high GI meals compared with the low GI meals. Therefore, visit was kept in the model. Gender was unrelated to performance, and as such removed from the model. When age, height, weight, and BMI were entered in the analysis with visit, these were not significantly associated with performance. Consequently, age, height, weight and BMI were removed from the model. Then, mood was entered in the analysis with visit, which explained more of the variance, resulting in a more significant GI effect ($F(1,59)=6.649$, $p=0.012$). Mood overrode the visit effect, and as such visit was removed from the analysis (GI effect: $F(1,60)=6.417$, $p=0.014$). Mood and the 'before minus baseline' glucose and cortisol levels were subsequently entered in the analysis, which similarly explained the variance and resulted in a significant GI effect ($F(1,54)=6.498$, $p=0.014$). Therefore it could be argued that these are just explanatory variables in the pathway between breakfast, mood and cognitive function. Finally, the glucose and cortisol values *per se* were entered in the analysis with mood. The addition of these variables removed significance, and it could be argued that the change in glucose and cortisol levels rather than the values *per se* partially explain the variance in this test.

Serial sevens ('correct')

The addition of visit did not explain any of the variance as a within-subject variable, and the overall GI, GL effect or GI*GL interaction remained non-significant. Therefore, visit was removed from the model. Gender was a significant between-subject factor, suggesting that boys performed better than girls on this task; as such it was preserved in the model, though the overall GI, GL effect or GI*GL interaction remained non-significant. When age, height, weight, and BMI were entered in the analysis with gender, height, weight and BMI explained some of the between-subject variance, while the overall GI, GL effect or GI*GL interaction remained non-significant. Consequently, age was removed from the model. Then, mood was entered in the analysis with gender, height, weight and BMI, which did not explain the variance. Therefore, mood was removed from the analysis. Gender, height, weight

and BMI and the 'before minus baseline' glucose and cortisol levels were subsequently entered in the analysis, which similarly did not explain any of the variance. Finally, the glucose and cortisol values *per se* were entered in the analysis. The addition of these variables overrode the effect of gender, height, weight, and BMI, which were removed from the model. This resulted in a significant GI effect ($F(1,67)=5.264$, $p=0.025$); that is, participants in the high GI meals performed better than participants in the low GI meals.

Delayed word recall ('correct')

The addition of visit explained some of the variance as a within-subject variable; participants performed better on their second visit compared with their first visit. The overall GL, GL effect and GI*GL interaction remained non-significant. Therefore, visit remained in the model. Gender was unrelated to performance, and as such removed from the model. When age, height, weight, and BMI were entered in the analysis with visit, it appeared that age *per se* was not as important as the remaining variables. The remaining variables (i.e. height, weigh and BMI) explained some of the variance as between-subject factors, though the overall GI, GL effect or GI*GL interaction remained non-significant. Therefore, age was removed from the model. Then, mood was entered in the analysis with height, weight, and BMI. The addition of mood did not explain the variance, and the overall p-value of the model was not significant. Height, weight, and BMI remained in the model as significant between-subject factors. Following that, the 'before minus baseline' cortisol and glucose levels were entered in the model with height, weight, and BMI; the addition of these variables did not explain the variance, while height, weight, and BMI remained in the model as significant between-subject factors. Similarly, the 'before' cortisol and glucose levels were entered in the model with height, weight, and BMI; similar findings were observed as for the 'before minus baseline' values. Therefore, for this task, height, weight, and BMI helped explain part of the between-subject variance (borderline: $F(1,69)=3.315$, $p=0.070$, $F(1,69)=4.019$, $p=0.049$, and $F(1,69)=4.210$, $p=0.044$, respectively), without affecting the overall GI, GL effect or GI*GL interaction.

In conclusion, the analysis revealed that gender was unrelated to performance, with the exception of the serial sevens task. Furthermore, visit order explained some of the findings related to within-subject variance (i.e. participants performed better on their second visit), and height, weight and BMI explained some of the between-subject variance (i.e. more developed children performed better). Nonetheless, these factors and covariates did not explain the variability observed (i.e. no significant GI, GL effect or GI*GL interaction came up for any of the main CF test variables). Variations in mood (i.e. change in mood) as a result of the meal administered had an impact that overrode other factors, that is visit effect, gender, height, weight, and BMI, and explained the variance for two out of the seven CF measures, resulting in a significant GI effect; the word generation, and the speed of information processing task. This effect was not observed simply by adding the baseline, 'before' or 'before minus baseline' glucose and cortisol values. For two of the tasks, Stroop and serial sevens, the absolute glucose and cortisol values explained the variance, resulting in a significant GI*GL interaction and a GI effect, respectively. Matrices and word recall (both immediate and delayed) performance were unrelated to GI, GL or GI*GL.

Therefore, evidence from the intervention study is consistent with the hypothesis for a GI but not a GL effect for the word generation task (i.e. low GI, better performance); and for a GL but not a GI effect for the Stroop task (i.e. high GL, better performance). On the contrary, evidence is not consistent with the hypothesis for a GI effect for the Stroop, speed of information and serial sevens task (i.e. high GI, better performance).

CHAPTER 5: DISCUSSION

5.1 Introduction

Three studies were conducted to answer the central question of this thesis: does the glycaemic potency of breakfast affect cognitive function and mood in adolescent school children? The first study, which was cross-sectional and not as robust as an intervention study was hypothesis generating, and intended to provide an indication of those associations that were worth exploring further. To date, no studies have been reported that have looked into the combined effects of GI and GL on CF. The cross-sectional study was unique in providing evidence in support of the assumption that GI and GL are associated with CF and mood in adolescents. This evidence justified the design and execution of a controlled intervention trial to test a more specific hypothesis, and to administer meals designed specifically to vary according to their macronutrient composition, GI and GL, reflecting the meals reported to have been eaten in the cross-sectional study. Based on that study, the hypothesis generated was that a low GI – high GL breakfast would be associated with improved CF and mood 90-120 minutes after breakfast. Furthermore, the cross-sectional study highlighted potential confounders and effect modifiers for further consideration in the intervention trial, including blood glucose levels, mood, socio-demographic characteristics, and inter-individual differences such as arousal, individual effort, motivation, anxiety/ stress, and perceived difficulty.

The aim of the second study was to investigate in a controlled setting the glycaemic, insulinaemic and cortisol responses of the breakfast meals that were reported eaten by the children in the cross-sectional study. The purpose of the study was to assess whether or not the theoretical differences in these blood parameters would be observed in practice. The study was unique in characterizing the glycaemic, insulinaemic and cortisol responses to the composite GI and GL meals. It also provided insight into the validity of the methods for calculating GI and GL of composite meals, being the first study of its kind to characterize the composite meals based on realistic glycaemic and insulinaemic responses, and not on computations of GI and GL. Moerover, to my knowledge, it was also the first study to investigate cortisol responses of meals differing in their GI and GL. The study also distinguished

between the two high GL meals (low or high GI), which had the same GL but differed in their energy content. Since the effects of the glycaemic potency could not be differentiated from the effects of energy content in the high GL meals, it was decided for a fifth meal to be included in the analysis (i.e. high GI – high GL), which would have the same energy and macronutrient composition as the low GI – high GL meal. The two low GL meals (low or high GI), had similar GL and energy content.

Therefore, the second study facilitated the selection of the two high GL meals (low GI, high GI) that would most differ in their glycaemic and insulinaemic responses. This proved to be the isocaloric high GI – high GL meal, M2b. Besides, this approach ensured iso-caloric experimental conditions within the GL groups (both low and high), which allowed detection of the impact on cognitive function of GI *per se*, rather than variations in energy. In addition, differences in energy content between the GL groups would allow detection of whether the effects can be generalised across GL and meal size. Furthermore, cereals were selected as the main CHO-sources for both the high and the low GI breakfast meals, as differences in the physical form of the foods selected (e.g. cooked breakfast/ toast *vs.* cereal) could have accounted for further variations in the glycaemic and hormonal responses of the participants, with subsequent effects on CF and mood.

Usually the glycaemic and insulinaemic responses are measured over a period of two hours. Since the final finger prick in the cross-sectional study was taken approximately 150 min (2.5 h) after breakfast, the time period was extended to 3 hours in order to characterize the response of these meals in both the immediate (0-2h) and the middle post-absorptive period (2-3 h). Cortisol was also measured to investigate whether there were gender differences in cortisol secretion, and whether GI and GL could have an effect on cortisol responses, due to perhaps meal differences in neuropeptide responses (Rohleder & Kirschbaum, 2007). The experimental situation (i.e. cannulation) would be unlikely to have an effect on cortisol levels, as the use of an intravenous catheter has been shown to facilitate sampling and reduce venepuncture related stress (King & Hegadoren, 2002). This study revealed that high GL meals had higher glycaemic and insulinaemic responses, while there were no differences in cortisol responses. Though not significant, within the same GL there

was a trend for the high GI meal to have higher glycaemic and insulinaemic responses compared with the low GI meal, with no differences in cortisol responses.

In brief, the previous two studies generated sufficiently clear outcomes to facilitate the characterization of the breakfast meals based on their glycaemic, insulinaemic and cortisol responses for the purposes of the intervention study. The aim of the intervention study was to investigate whether the breakfast meals previously tested in young adults, and which differed in their GI and GL, could produce differences in CF and mood in adolescent school children. An intervention trial provided a stronger basis to assess whether or not the energy, CHO and macronutrient composition of breakfast might affect CF and mood of young adolescents. Having the advantage that the meals used were already shown to differ in their glycaemic and insulinaemic responses and not in their cortisol responses, especially in the interval of interest (i.e. 90-150 min after breakfast administration), this study is the first of its kind to show accurately whether differences in the GI and GL of breakfast can bring about changes in cognition and mood as a result of the meals administered. The present study has provided useful insight into the possible effects of other confounders or effect modifiers, such as mood, glucose and stress levels (as measured by salivary cortisol levels). Finally, the primary hypothesis was tested and supported: a low GI – high GL breakfast is selectively associated with differences in cognitive performance and mood 90-120 minutes after breakfast administration.

5.2 Subjects

In the present PhD, adolescents 11-14 years old were selected to investigate the effects of the glycaemic potency of breakfast on CF and mood. There were possible implications of using 11-14 year olds, due to the potential variation in physiology and brain development. Adolescence is a critical period in the life of an individual, where emotional and cognitive changes take place, imperative for independent functioning. It is very difficult to put adolescence into chronological context, as there is no single event that indicates its onset or termination. Adolescence is usually placed between the ages of 11 to 18, without excluding the possibility that in some individuals it can extend into the early twenties. There is also evidence that the physical growth observed in adolescence is generally related to growth in cognitive abilities (Spear,

2000). A maturation of neurological processes takes place in relation to social and emotional behaviour and cognitive abilities. Although there is little increase in brain size after the age of five years, synaptic development clearly continues, resulting in adolescence in a major transformation of cognitive thought leading to age-related improvements in higher executive functions, such as abstract reasoning.

The widespread belief that cognitive abilities 'spurt' during adolescence should be interpreted with caution. It would be expected for some cognitive abilities to increase earlier in childhood and then to level-off during adolescence (i.e. primary functions, spatial orientation), and for others to increase linearly from childhood to adulthood (i.e. higher executive functions) (Gogtay *et al.*, 2004). Recent cross-sectional findings support this assumption (Waber *et al.*, 2007). Higher executive functions, such as coding matrix reasoning, block design, spatial working memory and passage comprehension improve in adolescence, while for other functions such as verbal fluency and verbal learning, adult levels of performance were approached between the ages of six to ten.

In light of this evidence, we would not expect puberty to be of greater influence in physiology and brain development than other periods of childhood, when the changes in brain development could be expected to be much greater. Adolescence is therefore no less good a time for investigating the relationship between food consumption and CF and mood than other periods of childhood, and no greater a source of variation in relation to development at a given age. Additionally, the selection of adolescents is pragmatic, and addresses the lack of data and well-designed studies looking into the effects of breakfast administration on cognitive function and mood in this age group. Finally, the fact that the effects of variations in the types of food consumed on CF and mood were detectable in adolescence suggests that variations in cognitive development in puberty are not so great as to prevent the observations of such effects.

To date, there have not been observed any differences in glycaemic responses between males and females (Wolever *et al.*, 2003). The study was not therefore designed to detect differences between sexes, and sampling of boys and girls was intended to increase the generalizability of the findings. The anthropometric measurements selected to characterize the sample were height and weight, and their

derivative, BMI (body mass index). The aim of taking anthropometric measurements was to help characterize the nutritional status (over and under-nutrition) of participating students. The raw values of height and weight can not easily be used in the anthropometric characterization of growing individuals, such as of our sample, as these are particularly age-dependent. BMI is a marker that evaluates an individual's weight status in relation to height (weight/ height^2) with weight in kilograms (kg) and height in meters (m). BMI for children, also referred to as BMI-for-age, is used for the assessment of both over and under-nutrition in childhood, and is gender and age specific (WHO, 2007). In 2007, the World Health Organization (WHO) recommended the use of BMI-for-age z-scores to differentiate overweight (+1SD) and obese (+2SD) boys and girls aged 5–19 years old (WHO, 2007). Recently, new cut-off z-scores have been proposed for thinness (Cole et al 2007); -1SD is considered as stage 1 thinness, -2SD stage 2 thinness (which was used as a cut-off in the present PhD), and -3SD stage 3 thinness. Low height-for-age is an anthropometric indicator of stunting; the cut-off points for the latter is -3SD (WHO, 2007). Both of these markers, BMI and height-for-age were used to characterise the nutritional status of our sample. Recent findings suggest that elevated BMI is not associated with cognitive function in healthy children and adolescents, though thinness could be associated with declined cognitive performance in girls (Gunstad *et al.*, 2008). Therefore, there is some evidence from the literature suggesting that BMI could be a confounder.

The reason why young adults (age range 18-30) were selected to measure the postprandial glycaemic, insulinaemic, and cortisol responses of the GI and GL breakfast meals was because it would be unethical to use children (i.e. a vulnerable population) for a study for which there is no direct benefit to the participants, and which was also quite invasive (i.e. cannulation required). Equal numbers of males and females were selected for the second study, again in order to capture gender effects on the postprandial responses. Taking into account that all the participants were healthy, had normal BMI, none of them had family history of diabetes, and that both their baseline whole blood glucose levels and their baseline venous plasma levels were <5.6mmol/L and <6.1mmol/L on all five visits (i.e. normal), respectively, it would be expected for the results to be comparable with healthy young adolescents (age range 11-14). To my knowledge, there has not been carried out any GI testing in children, or

any studies that have compared GI data or glycaemic, insulinaemic and cortisol responses in normal adults *vs.* normal children. The assumption has been made, therefore, that the findings observed here in young adults with regard to postprandial glycaemic, insulinaemic, and cortisol responses would be expected to be similar in adolescent children. Despite the fact that no studies have been carried out so far to investigate differences in glycaemic and insulinaemic responses between children and adults, it would be expected for the responses to be comparable in healthy populations with normal baseline glucose levels. Besides, the diagnostic criteria for diabetes are the same for both adults and children (WHO, 1999). With regard to cortisol levels, in adults these were measured in serum (unbound fraction), and in children in saliva (similarly unbound fraction). These two measures are known to correlate highly, although the absolute levels of cortisol in saliva are significantly lower than the ones in blood (Kirschbaum & Hellhammer, 1994). Besides, the functioning of the HPA-axis for cortisol is established in early infancy, and as such studies concerning HPA function in children are readily comparable to studies in adults (Genazzani *et al.*, 1983; Hanrahan *et al.*, 2006). Therefore, similar to glucose and insulin levels, the cortisol levels would be expected to be comparable between adults and children, so the young adult model in the second study was relevant to the intervention study in adolescents.

5.3 Glycaemic potency of breakfast and blood glucose, insulin and cortisol levels

The first thing that was investigated was whether the selected meals differed in their glucose, insulin and cortisol responses. Baseline glucose, insulin and cortisol levels can have an effect on both the exposure (i.e. glycaemic response) and the outcome (i.e. cognitive function, mood); therefore, these can be considered as genuine confounders. Differences in baseline glucose, insulin and cortisol levels reflect true differences in physiologic responses between subjects, which are of course expected to be highly variable. It was clear in the analysis that when these baseline individual physiological variations were taken into account by assessing the 'minus baseline' values, they were not independently responsible for the observed effects. Results from all three studies provided findings that allowed the characterization of the meals. For all three studies and all physiological measurements, the main time-points of interest were 90 min after breakfast (i.e. first finger prick or saliva collection), ~150 min after

breakfast (i.e. second finger prick or saliva collection), and the difference between these two time-points (i.e. BG at 150 min minus BG at 90 min). The clinical (second) study provided the advantage of characterizing the meals at many time-points and also based on their glycaemic and insulinaemic reponses (i.e. iAUC 0-2 and 2-3 h).

Blood glucose (1st, 2nd, and 3rd study)

It was hypothesized that after a high GL breakfast meal BG levels would not have dropped below baseline 90 minutes after breakfast, while this would be the case after a low GL meal. For a given GL, the low GI meal was expected to maintain glucose levels more efficiently than the high GI meal (i.e. lower for longer). In the high GL groups, a slower drop in BG levels was expected after the low GI compared with the high GI meal; conversely, for the low GL groups, a quicker recovery to baseline was expected after the low GI compared with the high GI. The mental demand was not expected to have an impact on BG levels.

The cross-sectional nature of the first study cannot confirm the previous hypothesis; nonetheless, the following observations were consistent with it. First, the distribution of the difference in BG levels during the testing showed that half of the children's BG increased and for half of them it dropped. Furthermore, a drop was observed in the high GL groups (when participants were distributed in two GL groups), and a rise in the low GL groups (significant). This means that a quicker drop was observed in the high GL groups. On the contrary, the GI effect was not significant.

The findings from both the clinical study in adults and the intervention study in children can be used to support the previously stated GI, GL and BG hypothesis. In the clinical study in adults, two analyses were available, A and B. When either analysis A (similar meals as in cross-sectional study) or B (same meals as in intervention study) were used to investigate the GL and GI effects during the 90-150 time interval, both the GL and GI effects were significant (Analysis A: $F(1,8)=10.940$, $p=0.011$, borderline: $F(1,8)=5.070$, $p=0.054$; analysis B: $F(1,8)=12.494$, $p=0.008$, $F(1,8)=6.169$, $p=0.038$). Specifically, a bigger drop was observed in the high GL meals compared with the low GL meals (in the low GL groups the difference was close to zero), and a bigger drop in the high GI meals compared with the low GI meals. Figure 4.4 (page 157) and Figure 4.12 (page 177)

depict these significant effects. Therefore, in the high GL groups BG levels declined during the 90-150 min interval (but BG levels were above baseline), and the drop was greater in the high GI meals (as expected, i.e. low GI meals preserved BG levels). In the low GL meals, the drop was smaller compared with the high GL meals (but BG levels were below baseline), and similarly the drop was greater in the high GI meals (Mean values (±se): M1: -0.50 (0.19); M2a: -1.04 (0.33); M2b: -1.00 (0.20); M3: -0.05 (0.15); M4: -0.33 (0.22)). From Figure 4.4 it can also be seen that the low GI – high GL meal preserved the glucose levels above baseline for the entire three hours after breakfast. Thus, the original hypothesis that the high GL meals would be associated with BG levels above baseline, and the low GL meals with BG levels below baseline was confirmed. Furthermore, the hypothesis that the low GI meals would preserve BG levels was also confirmed. These findings apply when stress is not present, and there are not any potential cortisol-induced effects on BG levels.

The clinical study in adults confirmed previous observations that GI alone is unable to predict the glycaemic responses when different amounts of carbohydrates are eaten, and supported the use of GL (Wolever *et al.*, 2006; Galgani *et al.*, 2006). Among 12 obese female subjects, differences were observed in the iAUC glucose only between a large high GI meal (i.e. M2a, M2b) and a large low GI meal (i.e. M1), and not between a small high GI meal (i.e. M4) and a small low GI meal (i.e. M3) (Galgani *et al.*, 2006). Nonetheless, the differences in GI between the low and the high GI meals were almost double (GI: 43 *vs.* 85, differed by 42 GI units), while in the present study the low GI meals were 1.3 times smaller in GI compared with the high GI meals (GI: 48 *vs.* 61, differed by 13 GI units). However, the meals selected in the Galgani study were unrealistic, and could not be consumed in real-life conditions, contrary to the ones selected in the present study. Therefore, the reason why a GI effect (though observed) was not significant could be attributed to the not big enough differences in GI between the low and the high GI meals, or to the few observations (only ten participants); still, even a two-fold difference in GI failed to produce significant GI effects in the low GL meals in the Galgani study. Overall, GL was a strong predictor of the glycaemic responses over a period of three hours after the ingestion of the meals. High GL meals predicted higher glycaemic responses compared with low GL meals. Glycaemic index failed to predict iAUC glucose in the early (0-2 h) and the middle (2-3h) post-absorptive period. Nonetheless, the GI effects were in the expected

direction: in the early post-absorptive period high GI meals were associated with higher glycaemic responses, while in the late post-absorptive period low GI meals were associated with higher glycaemic responses.

The intervention trial in children also provided proof in support of the assumption that meals differing in their GI and GL produce differences in glucose responses. The following table compares the results from the clinical study in adults (analysis B) with the ones from the intervention study in children. Time zero (i.e. average time of breakfast administration) was similar in both studies (08:46 am, and 08:55 am, respectively); therefore, the actual time BG was measured at the different time-points is comparable between the two studies. In the results, it was demonstrated that there were no differences in the time BG was measured between the four GI and GL meals, both for the study in adults (see section 4.2.1, page 140) and the study in children (see section 4.3.2, page 172). Therefore, the testing was accurately timed and the results are comparable not only within the same study (i.e. in adults or in children), but between the two studies as well (i.e. adults and children).

	GLUCOSE LEVELS	
TIME-POINTS	**STUDY IN CHILDREN**	**STUDY IN ADULTS**
Baseline	GL*GI interaction: in the low GL: Lo GI>Hi GI	None
90 min ('minus baseline')	GL & GI effect: Hi GL>Lo GL Hi GI>Lo GI	GL effect: Hi GL>Lo GL
150 min ('minus baseline')	GL effect: Hi GL>Lo GL	GL effect: Hi GL>Lo GL
150 min minus 90 min (i.e. 'after minus before')	None but observed GL & GI trend: Hi GL>Lo GL (bigger drop) Hi GI>Lo GI (bigger drop)	GL & GI effect: Hi GL>Lo GL (bigger drop) Hi GI>Lo GI (bigger drop)

In the intervention study in children, there was a significant GI*GL interaction, suggesting that the 'minus baseline' measures would be more appropriate to investigate the effects of the GI and GL on BG levels. At both 90 min (i.e. before the CF tests) and 150 min (i.e. after the CF tests) there was observed a significant GL effect, where the high GL meals were associated with higher glucose levels compared with the low GL meals. The lack of significance of GI in the adult study –as explained

before– could be attributed either to the small number of subjects (10) or to the small GI difference between the low and the high GI meals within the same GL. The fact that there were not detected statistically significant GI effects with regard to the glucose levels does not necessarily mean that there were not in fact differences in the physiologic responses between these meals. Figure 4.4 depicts GI differences between these meals, which were in the expected direction; that is, within the same GL the high GI meal was associated with the higher blood glucose levels. Furthermore, in the 3rd and final study where the number of subjects was greater (n=74), there were in fact GI differences 90 minutes after breakfast consumption (the high GI meals higher BG levels than the low GI meals), which is similar to what was observed in the study in adults (but did not reach significance). The GI effect did not reach significance for the 150 minute time-point, but the observed trend for both studies was for the low GI meals to be associated with higher glucose levels compared with the high GI meals. Besides, 2.5 h hours after a meal, it would be expected for the BG levels to be returning to baseline levels (thus, justifying the non-significant effects).

During the testing (i.e. 150 minus 90 min interval) the GI and GL effect was significant for the study in adults; the high GL meals and the high GI meals were associated with a bigger drop in glucose levels compared with the low GL and low GI meals, respectively. The same trend was observed in the intervention study in children. Figure 4.11 (children, page 177) and Figure 4.12 (adults, page 177) clearly illustrate that these four meals followed exactly the same pattern in both studies, although in the high GI – high GL meal responses appeared to be suppressed in children compared with the adults. The reason for the latter remains unclear. Therefore, GI and GL predicted the glycaemic responses in both studies, and it can furthermore be argued that the experimental situation (i.e. CF testing) did not have an effect in glucose levels over and above that brought about by meal administration, contrary to what has been suggested so far; that is, that during the testing a drop in glucose levels should be observed reflecting glucose uptake by the 'working' brain.

Insulin (only 2nd study)

Insulin was assessed only in the clinical study in adults, as it cannot be measured in capillary blood. The significant GI and GL effects on insulin levels for the time-points of interest are presented in the following table:

	INSULIN LEVELS
TIME-POINTS	**STUDY IN ADULTS**
Baseline	GI effect: Lo GI>Hi GI
90 min ('minus baseline')	GL effect: Hi GL>Lo GL
150 min ('minus baseline')	GL effect: Hi GL>Lo GL
150 min minus 90 min (i.e. 'after minus before')	GL effect: Hi GL>Lo GL (bigger drop)

From this table it can be seen that high GL meals predicted higher insulin levels compared with low GL meals, 90 and 150 min after breakfast. When the 90-150 min interval was assessed there were only significant GL effects (i.e. bigger drop in the high GL meals; analysis A: $F(1,8)=14.057$, $p=0.006$; analysis B: $F(1,8)=22.701$, $p=0.001$), and no significant GI or gender effects. Overall, GL was a strong predictor of the insulinaemic responses over a period of three hours after the ingestion of breakfast meals differing in their GI and GL. High GL meals predicted higher insulinaemic responses compared with low GL meals. Glycaemic index failed to predict iAUC insulin in the early post-absorptive period, but predicted iAUC insulin in the middle post-absorptive period. Specifically, in the early post-absorptive period there were not any differences between the low and the high GI meals, while in the middle post-absorptive period the high GI meals had higher insulinaemic responses compared with the low GI meals.

Cortisol (2nd and 3rd study)

Similar to glucose levels, the results between the study in children and the study in adults are comparable since time zero (i.e. average time of breakfast administration) was similar for both studies (08:46 am, and 08:55 am, respectively). In the results, it was demonstrated that there were no differences in the time these measures were taken between the four GI and GL meals, for both the study in adults (see section

4.2.1, page 140) and the study in children (see section 4.3.2, page 172). Therefore, the testing was accurately timed and the results are comparable both within the same study (i.e. in adults or in children), and between the two studies as well. Furthermore, the baseline cortisol levels on the testing days (i.e. 'stressful' occasions) were compared with the baseline levels on a normal day where no testing took place (i.e. 'stress-free' occasion). This suggests that at baseline, the children were no more stressed on the testing days than on any other typical day. The following table presents the significant GI and/ or GL effects at the time-points of interest.

	CORTISOL LEVELS	
TIME-POINTS	**STUDY IN CHILDREN**	**STUDY IN ADULTS**
Baseline	GI effect: Lo GI>Hi GI	None
90 min ('minus baseline')	GI effect: Lo GI>Hi GI (bigger drop)	None
150 min ('minus baseline')	GI effect: Lo GI>Hi GI (bigger drop)	None
150 min minus 90 min (i.e. 'after minus before')	None but observed GL & GI trend: Hi GL>Lo GL (bigger drop) Lo GI>Hi GI (bigger drop)	None

One could argue that participants were not aroused enough by the experimental situation, and this is why no changes were observed in BG levels during the testing over and above those brought about by the meal administration. The present findings confirmed that this is not the case. First of all, the study in adults confirmed that there were no differences in cortisol responses between the four GI and GL meals that were later used in the intervention study in children. The intervention study in children revealed that there were differences in cortisol responses between the four groups, which effectively could not be attributed to the meals, but to the experimental situation (i.e. CF testing). Therefore, this finding confirms that the children were aroused by the testing. The only thing that remains to be elucidated is whether the cortisol responses differ based on the meal administered. Indeed, at both 90 and 150 minutes ('minus baseline' values) after breakfast a GI effect was observed; low GI meals were associated with a bigger drop in cortisol levels (i.e. high GI meals, higher cortisol levels). During the CF testing (i.e. 150 minus 90 min interval), the observed

trend was for the high GL and the low GI meals to be associated with a bigger drop in cortisol levels. Cortisol levels fall progressively throughout the morning. Therefore, since the high GI meals were associated with higher cortisol levels compared with the low GI meals, it could be argued that the low GL (not significant), low GI meals are associated with better response to stress (i.e. lower cortisol levels). Whether the latter meal effects on cortisol responses can affect performance or mood is going to be discussed in the next section.

5.4 Glycaemic potency of breakfast and cognitive function

It was hypothesized that a low GI meal would minimize glycaemia fluctuations and facilitate performance and mood for longer following breakfast consumption compared with a high GI meal; and that a high GL would potentiate the glycaemic potency of the meal. Therefore, based on this hypothesis it would be expected for the lower glycaemic response within the high GL meals, that is the low GI – high GL, to be beneficial (i.e. intermediate glycaemic response compared with all other meals), and not for the lowest glycaemic response (i.e. high GI – low GL) to be beneficial.

With regard to glycaemic responses, there should be a distinction between a low glycaemic response as determined by both GI and GL (which is the recommended approach) and a low glycaemic response as determined solely by GI or GL. The literature to date in general predicts that a low glycaemic response is beneficial, but it does not distinguish between a high, intermediate, and a true low glycaemic response (i.e. the lowest among the meals compared when both the GI and GL are taken into account). This lack of distinction can be attributed to the fact that none of the studies in this field have looked into both GI and GL or measured the glycaemic responses of the meals to describe fully the glycaemic response. Therefore, when a low glycaemic response is suggested as beneficial by other studies in this field, this should in fact be interpreted with caution, as there are methodological problems surrounding these studies (reviewed below). This is why there are a lot of inconsistencies in the findings reported in the literature.

It is only recently that the use of GI or GL (but not together) has been considered as a measure against which to assess the effects of CHO-containing foods or meals on cognitive function. In 71 healthy young adults (mean age: 21±1, all females), a low GI plain biscuit (n=36, GI=42.3, 229.5kcal), but not a high GI breakfast cereal bar (n=35, GI=65.9, 219.5kcal) was associated with improved performance on the 'difficult' abstract words but not the 'easy' concrete words of a **word list recall task** (the only task administered) 150 and 210 min after breakfast, but not earlier (Benton *et al.*, 2003). These foods were served with a sugar-free orange drink, decaffeinated coffee or tea with added skimmed milk and artificial sweetener (if required); as no information is provided by the authors with regard to the nutrients of these products or how much milk was added, it is impossible to estimate accurately the overall GI of the meal. Nonetheless, both the GI values and the total energy would be very close to the ones reported (low GI: 42.3, 229.5 kcal; high GI: 65.9, 219.5 kcal), as the products the foods were served with do not have a GI value (with the exception of milk). Based on the reported values, the estimated GL of the food is 14.5 for the low GI and 20.6 for the high GI (for calculations see section 2.3.2, page 59). Such small differences in GL are unlikely to account for the findings.

A study in school children aged 9-11 years (15 males, 15 females) and 6-8 years (15 males, 15 females) used a cross-over design in which subjects consumed either instant oatmeal (low GI), ready-to-eat cereal (high GI), or no breakfast (Mahoney *et al.*, 2005). **Spatial memory** (i.e. map task) and **short-term memory** (i.e. digit span task) (in girls only) in both age groups and **auditory attention** (i.e. vigilance) in 6-8 year olds were improved one hour after the low GI breakfast compared with the high GI or the no breakfast. The breakfast cereals were served with half a cup of skimmed milk and both provided 200kcal as energy. The authors provided no information on the GI of the individual foods, or the overall GI of the breakfast meals. If it is considered that the oatmeal has a GI of 66 (International Table of GI and GL values, entry 216), the ready-to-eat cereal a GI of 80 (which is an average value of a high GI breakfast cereal that provides 417kcal and 83g of CHO per 100g) and the skimmed milk a GI of 32 (International Table of GI and GL values, entry 373) then the low GI meal would have a GI of 60.6 and a GL of 23.0, and the high GI meal a GI of 72.0 and a GL of 25.9. So, the GL of the meals is about the same. Therefore, both studies above

considered GI but not GL, as they kept the energy and macronutrient content of the two breakfasts the same.

Ingwersen (Ingwersen *et al.*, 2007) found that in 64 children aged 6-11 (26 boys, 38 girls) there was less decline in **accuracy of attention** and **secondary memory** two hours following a low GI breakfast (All Bran, GI=42) compared with a high GI breakfast (Coco Pops, GI=42). Both cereals were served with 125ml of semi-skimmed milk. Therefore, the meal GI and GL can be calculated as 37.2 and 8.3 for the low GI meal and, 68.0 and 24.5 for the high GI meal, respectively. The observed differences were attributed by the authors to the GI, while GL was not controlled for. Moreover, the macronutrient composition of the meals was different (High GI cereal: 133 kcal, Low GI cereal: 98 kcal; 36% difference in energy alone). To test if GI alone is having an effect, the nutritional (fat, protein, CHO) composition of the meals should be kept the same (or similar), and only the CHO source should be varied. Therefore, in this study other factors could be responsible for the observed effects.

Nabb and Benton (Nabb & Benton, 2006) tested eight breakfast meals (main source of CHO: crispbreads) designed to offer various combinations of high and low levels of CHO, protein and fat, in young adults aged 20-25 years. Memory (immediate and delayed), vigilance (rapid information processing task) and reaction times were assessed 30, 75 and 120 min after breakfast administration in 189 young females. The observed effects depended on individual differences in glucose tolerance (participants were classified in two groups: ≤5mmol/L better glucoregulators, n=64; >5mmol/L poorer glucoregulators, n=125). The study was not designed to investigate the effects of GI or GL, and it was unbalanced. Though this study was not designed to investigate the effects of either GI or GL, there was one interesting interaction between glucose tolerance, glycaemic load and both reaction times and vigilance; only in people with better glucose tolerance, a low GL breakfast was associated with **slower decision times** and **worse vigilance** compared with a high GL breakfast. Furthermore, vigilance performance was better after a low GL breakfast, but in people with poor glucoregulation.

A recent study assessed the effects of GL on the behaviour of 19 children (ten females, nine males) aged 6-7 years old (Benton *et al.*, 2007). Besides behaviour (not specifically of interest here), memory (i.e. recall of objects) and ability to sustain attention was measured two and a half hours after breakfast administration. Three breakfast meals were administered that differed in their physical form (i.e. not all cereal-based meals) and their macronutrient composition (high GL=39.0, GI meal=70.2, 305kcal; medium GL=14.8, GI meal=54.0, 284kcal; low GL=5.9, GI meal=50, 299kcal); this effectively means that although these meals had similar energy content they differed not only in their CHO content as it would be expected from the different GL, but also in protein and fat content. The medium and low GL meals were very similar in all nutrients apart from CHO, while these differed considerably compared with the high GL meal. There were no meal effects or interactions with regard to **memory** (i.e. immediate recall, spatial memory, delayed recall) or the ability to **sustain attention.**

In general, in the Mahoney study, out of the six measures, visual perception, visual attention, and verbal memory (recall) were not affected, while spatial memory, short-term memory and auditory attention were. In the Ingwersen study 11 tasks were used, which were then combined into five CF domains. Only two domains were affected (accuracy of attention and secondary memory); speed of attention, speed of memory, and working memory were unaffected. In the Benton study (2003) verbal fluency (i.e. word list recall) was affected, while in the Benton study (2007) memory and the ability to sustain attention were not affected. In the Nabb study decision times and vigilance were affected. From these studies it emerges that vigilance (i.e. attention) seems to be consistently associated with either low GI or high GL (though there is one inconsistency with the Benton study (2007)). There are inconsistencies with regard to all the other measures, especially with regard to verbal memory and memory recall. One would expect that these measures would be affected by low GI and or/ high GL, as glucose has been shown to improve both these domains (Foster *et al.*, 1998; Sunram-Lea *et al.*, 2001). Nonetheless, it should always be kept in mind that a CHO-rich breakfast may not produce the same effects on CF and mood as glucose does; a CHO-rich breakfast will be different in terms of absorption rates, gastric emptying, metabolic effects and secondary hormonal responses. In addition, the tasks selected between the studies are not exactly the same, therefore, lack of sensitivity of the task,

rather than lack of an effect could be responsible for the inconsistencies. Another important observation that emerges is that results appear to differ according to whether GI or GL is measured. Specifically, a low GI meal has been found to improve performance on a word recall task (Benton *et al.*, 2003), and on a spatial memory, short-term memory and auditory attention task (Mahoney *et al.*, 2005). On the other hand, a low GL breakfast was associated with slower decision times and worse vigilance in people with good glucoregulation (Nabb & Benton, 2006). This makes the need for well-balanced studies that investigate the combined effect of GI and GL on cognition imperative.

A recent review in this area of research concluded that there are three major outcomes when considering the effects of acute macronutrient administration on CF in young adults; first, that memory appears to be the domain most consistently affected; second, that mentally demanding tasks seem to be particularly sensitive; and third, that tasks sensitive to glucose administration include the serial sevens task, free word recall, cued word recall, and the word generation task (Hoyland *et al.*, 2008). Despite the majority of the observed effects being associated with memory tasks, the authors concluded that a wide range of tasks within a certain domain should be employed, before it can be argued that a particular macronutrient has or does not have an effect. In the present section, these three primary outcomes will be considered in relation to the findings from the cross-sectional and the intervention study in children.

In the present research, seven cognitive function tests were used: word generation task, immediate word recall, Stroop task, matrices, speed of information processing, serial sevens, and delayed word recall. It can be argued that these tests fall into three separate domains in order to be able to more clearly understand the findings from the cross-sectional and the intervention study: memory (word generation task, immediate and delayed word recall), vigilance (Stroop task, speed of information processing and serial sevens), and reasoning (Matrices). It has to be noted here that serial sevens task is an attentional task (i.e. vigilance) that requires use of working memory for its successful completion; it assesses both numeric working memory and vigilance. Therefore, also considered is whether facilitation of performance was being driven by working memory or vigilance.

In the cross-sectional study, participants were classified in four GI and GL groups based on their reported breakfast, in order to study the effects of the glycaemic potency of breakfast on cognitive function and mood. This classification was based on the median for GI and GL, and it yielded statistically significant results for the majority of the CF tests used in this study. Furthermore, it produced four well matched groups, as there were no differences in the distribution of age, height, weight, BMI-for-age, Hb, usual breakfast consumption, ethnic group and SEG. Exposure misclassification can not be ruled out, but that would have attenuated the results to the null. The fact that significant associations between GI and GL and CF were observed suggests that there are true associations between the glycaemic potency of breakfast and CF; the direction of these associations would be determined in the intervention study. The mean GI and GL of the low GI – high GL breakfast was 53 (se=1.4) and 43 (se=3.2), respectively. To our knowledge, there are no data regarding the GI or the GL of the diet or breakfast of healthy school children in the UK. In a recent study, the GI of the diet of German children aged 7-8 years was determined; the average GI of their diet 56.5 (sd=3.4) is in line with the mean GI of the breakfast reported in the present study 57.6 (sd=11.8) (Buyken *et al.*, 2005).

In the cross-sectional study, correlation analysis revealed that there were no linear associations between the GI or the GL of the breakfast and CF when entered as continuous variables. Multiple regression analysis confirmed this finding. This may in part be explained by an inverted U-dose response curve that exists between glucose and CF (Parsons & Gold, 1992; Flint, Jr. & Turek, 2003), which would yield low or non-significant correlations, and further supports the classification of participants into four groups based on both the GI and the GL content of the breakfast. Glycaemic index can be viewed as a measure of the 'potential relative increase in glycaemia' (i.e. to glucose or white bread) and GL of the 'potential absolute increase in glycaemia' (Buyken *et al.*, 2007). The use of both provides a better measure of the glycaemic potency of a given food or meal. Besides, when students in the present study were divided into low and high GI and GL groups, based on the median for these measures, performance on the CF tests appeared to improve for the low GI compared with the high GI, and for the high GL compared with the low GL. This observation further supported the classification of children into four groups, based on both the GI and the GL.

The main finding in the cross-sectional study was that the low GI – high GL (i.e. effect of both GI and GL) breakfast was associated with better outcomes measured 90-120 minutes later on two CF tests (serial sevens and speed of information processing), reported by the participants to be the most mentally demanding of the tests administered. This finding is consistent with previous studies claiming that in order for an effect of glucose on cognition to be observed, the tasks need to be sufficiently mentally demanding (Owens *et al.*, 1997; Donohoe & Benton, 1999a; Kennedy & Scholey, 2000; Scholey *et al.*, 2001; Benton & Nabb, 2003). Although it is difficult to quantify cognitive demand, the duration of the task, its complexity and time pressure probably all contributed to the ratings of cognitive demand obtained in the present study. Similarly, though a task could be viewed as 'simple', such as the speed of information processing task (so, theoretically not demanding) it could truly involve complex cognitive processes or the time pressure might have contributed to its classification as demanding. Therefore, the phrase 'mentally' demanding rather than 'cognitively' demanding is going to be used, to broaden this concept slightly. Besides, any characterisation of the tasks as demanding or not was based entirely on how these were rated by the participants. It is apparent that there is a need for clear definitions and standardization of the classifications of tasks as 'simple' or 'complex' or even 'demanding' among studies of this kind.

The addition of covariates improved the model for two more tests, the immediate word recall and the matrices. Specifically, higher GI (but not GL) was associated with better performance on the immediate word recall, and higher GL (but not GI) with better performance on the matrices. These tests were not ranked by the participants among the most difficult tasks. Therefore, it could be argued that it is not the mental demand that drives the GI and/ or GL effect, but the cognitive domain. Another observation that emerges from this study is that GI seems to differentially affect different cognitive domains; low GI was associated with improved performance on two vigilance tasks, but worse performance on a memory task. The reason for this remains unclear. It could be that an interaction takes place between glucoregulatory processes, arousal and subsequent cortisol secretion (the mechanisms will try to be elucidated in the intervention study). On the other hand, higher GL was consistently associated with better performance.

Therefore, four out of the seven CF measures were affected: short-term memory (i.e. immediate word recall), inductive reasoning (i.e. matrices), and sustained attention (i.e. speed of information processing task, serial sevens). Serial sevens task is an attentional task that requires use of working memory for its completion. The fact that both the speed of information processing task and the serial sevens task were affected by GI and GL in the same way, suggests that these two tasks assess the same domain (i.e. vigilance, how quickly information is being processed). This could be attributed to the fact that more emphasis was put on the participants to complete these tasks as quickly as possible. On the other hand, verbal fluency (i.e. word generation task, memory), long-term memory (i.e. delayed word recall), and selective attention (i.e. Stroop task) were not associated with either GI or GL.

This cross-sectional study is the first of its kind to show that not only GI, but also the total CHO content of the meal together with its rate of absorption (as indicated by GL) are factors affecting cognitive outcome. The intervention study, due to its nature, did not have the limitations of a cross-sectional one (i.e. misclassification, recall bias) and allowed for causal associations to be investigated. In the results section, it was revealed that when only GI and GL were entered in the repeated measures analysis without any other factors and covariates, there were not many GI or GL associations with performance. This is perhaps not surprising, considering that there were likely to be other mediating factors affecting the outcome that could be a direct outcome of the meal administered. Among these, the most important ones were glucose and cortisol levels, and of course mood. The addition of mood, and/ or glucose levels explained variance for four out of the seven CF measures used in this study, and for the main CF test scores' variables. Therefore, it could be argued that fatigue (i.e. the later tests only being affected) seemed not to be driving any observed effects between GI and GL and performance. Mental load (i.e. more demanding tasks) was not possible to assess in this study as there were differences in which tasks were reported to be more difficult between the low and the high GI meal (see section 4.3.4, page 185). Besides, since four out of the seven measures were affected it is unlikely that any observed effects could be attributed to mental load (similar to cross-sectional findings). Furthermore, facilitation appeared to be specific to certain parameters of the tests (i.e. 'correct' responses), probably because there were not many incorrect responses. Matrices performance was unrelated to GI and GL possibly because it reflects acquired

information and learned material over a longer period of time, leaving little potential for learning, and for a short-term impact of GI and GL to take place. There are two reasons why word recall (immediate and delayed) was not related to either GI or GL: either because the task was not sensitive enough to detect differences in GI and/ or GL (i.e. perhaps the words selected were not as difficult), or because height, weight and BMI as markers of physiological maturity explain any variance. In support of the former assumption are the findings from Benton (Benton *et al.*, 2003), who reported an effect of GI only for the most 'difficult' abstract words, but not for the 'easy' concrete words. The words selected in the word recall task used in the PhD studies were of similar difficulty; therefore, it is not possible to investigate such differences. Besides, previous studies looking into glucose administration and cognitive function have not shown an effect on this task (Foster *et al.*, 1998; Sunram-Lea *et al.*, 2001; Green *et al.*, 2001)

The story that seems to be emerging is that the effect of GI is domain specific. A low GI meal was associated with better performance on the word generation task (memory domain), when mood and glucose and cortisol levels were considered. The low GI meals were shown to be associated with feeling less 'nervous' and 'happy' and with lower glucose and cortisol levels ('before minus baseline' measures). Therefore, it could be argued that lower BG levels as a result of a low GI meal can facilitate memory performance, which is in agreement with previously reported findings (Nabb & Benton, 2006).

A high GI meal was associated with better performance on three tasks: Stroop task ('control'), speed of information processing, and serial sevens. The reason why the 'control' and not the 'actual' task of the Stroop was affected could be because GI might selectively facilitate performance that has to do with solely how quickly information is being processed (i.e. 'control' task), and not with response inhibition (i.e. 'actual' task). It could also be that in the 'actual' task there was a speed-accuracy trade-off, that is, participants sacrificed speed at the expense of accuracy (which is reflected by the few incorrect responses). This observation, that the apparently most difficult Stroop trial was not affected, further supports the assumption that cognitive demand does not seem to be an issue here. Serial sevens performance was facilitated by a high GI meal indicating that in this task how quickly information was being

processed (i.e. speed-accuracy trade-off) was driving the effect, and not working memory, where we would expect perhaps a low GI meal to have a facilitating effect. The absolute glucose and cortisol levels explained variance for the Stroop and serial sevens task, rather than mood and the change in glucose or cortisol levels, which explained variance for the speed of information processing task. This could be because they may represent slightly different areas or combinations of CF. In these tasks, the GL of the meals was only related to Stroop task performance, where the GI effect was only observed in the high GL group. This is in agreement with previously reported findings, where higher BG levels were associated with better vigilance (Nabb & Benton, 2006).

The size (i.e. energy content) and macronutrient composition of the breakfast were unrelated to the CF test scores and the BG levels, as assessed by correlation and adjusted regression analysis. It could be that in high CHO meals, such as the ones reported in this study, GI and GL are more influential compared with the absolute size of the meal or its macronutrient composition. Besides, the distribution of participants in four GI and GL groups would allow for possible associations between GI and GL and CF, BG, and mood to be more easily observed.

There were inconsistencies between the pilot study and the intervention study with regard to the GI and GL effect on cognitive performance. These observed inconsistencies can be attributed mainly to the cross-sectional nature of the first study and to the fact that breakfast was recorded and not administered. This could have resulted in errors in GI and GL calculations, which could have led to over or under-estimation of these measures. It could also be attributed to differences in social class or learning environment not adequately controlled for in the analysis (i.e. residual confounding). Despite the fact that there was available a measure of social class, this was based entirely on occupation, and did not include information about income or learning environment at home, which could have greatly confounded the results. Nonetheless, the cross-sectional study served its purpose, which was to reveal GI and GL associations, and to highlight potential confounders. The direction of these associations was revealed in the intervention study.

Despite these inconsistencies, both of these studies confirmed that there were no gender differences, with the exception of the serial sevens task. This is not surprising considering that boys perform better on tests of arithmetic cognition than girls (Carr & Davis, 2001; Rocha *et al.*, 2005). Furthermore, these studies confirmed that usual breakfast consumption was unrelated to performance, probably because the majority of the students had breakfast every day in both studies; and that age, height and weight can be considered as a proxy of maturity and physical development, and are positively correlated with performance. The intervention study, more important, suggests that changes in mood are important predictors of performance on CF tests.

5.5 Glycaemic potency of breakfast and mood

As for cognitive function, results from both the cross-sectional and the intervention study are going to be considered here. The cross-sectional study allowed only for associations to be investigated, and not for effects (i.e. causal inference). The mood scales before the CF tests were regarded as a potential predictors of performance on the CF tests, while the mood scales 'after' as an outcome of both the CF testing and the meals eaten at breakfast. In the cross-sectional study, positive moods before the CF tests such as 'happy', 'friendly', 'relaxed', and negative moods 'angry', 'sad', 'dissatisfied', were associated with lower scores. Furthermore, feeling 'nervous' before the tests was associated with higher scores. Similarly, after the CF tests positive moods 'lively', 'friendly', 'calm', 'happy', 'contented' were negatively associated with performance, and negative moods such as 'drowsy', 'sluggish', 'tired' were positively associated, while 'sad', 'dissatisfied', 'uncertain', and 'muddled' were negatively associated with performance. One possible explanation of these findings is that the students who felt more 'friendly' and 'happy' both before and after the CF tests were probably feeling relaxed and friendly towards the researcher and were not motivated/ aroused enough by the testing or the mental load. Furthermore, the negative moods that were associated with lower scores imply that perhaps the child was not very keen/ happy with his/ her participation in the study ('before') or unsure/ disappointed with his/ her performance on the tests ('after'). The positive association with 'nervousness' 'before' and performance implies that the children were aroused by the experimental situation, which in turn enhanced their attention and response. The negative moods after the CF tests that were positively associated with

performance suggest that the students were probably feeling worn-out/ tired after trying hard to perform well on the tests.

Feeling 'friendly' before the CF tests was the mood state more consistently significantly associated with the CF test scores' outcomes. Therefore, it was entered as a covariate along with other predictors in univariate analysis for each one of the CF tests. Performance on both immediate and delayed recall was predicted by feeling less 'friendly' before the CF tests; that is the more 'friendly' the participants felt the worse they performed on the word list recall task. This could perhaps reflect, that the children who reported feeling 'friendly' were not aroused by the experimental situation, and therefore performed poorly. Students in the low GI – high GL group reported feeling the least 'friendly' and 'calm' compared with the other groups.

Mood can be affected by meal administration. In the cross-sectional study there were observed differences between the size and the macronutrient composition of the meals, GI and GL, and mood. Specifically, higher energy and macronutrient intake at breakfast were associated with feelings of tenseness (i.e. more 'tense', less 'calm') both before and after the CF tests, which could effectively represent a state of arousal/ alertness. Furthermore, the high GL meals were associated with feeling more 'tense' and 'uncertain' and less 'calm' and 'contented', which similarly represents a state of arousal. This is in agreement with the general argument that a low fat – high CHO meal decreases fatigue and increases alertness compared with a high fat meal, when protein is kept constant (Gibson & Green, 2002). In the present study, the meals did not differ in their percentage contribution of protein to energy, while they differed in their contribution of CHO and fat to energy. The high GL meals had higher percentage contribution of CHO to energy and lower percentage contribution of fat to energy compared with the low GL meals. Therefore, it could be argued that feeling more 'tense' and less 'friendly', 'calm' and 'contented' may represent a state of arousal and alertness in the high CHO meals.

In the intervention study, the rate of change in mood from baseline to before the tests (i.e. net effect of meal administration), rather than mood *per se* immediately before the tests (i.e. a result of both meal administration and mood at baseline) was shown to be consistently associated with GI and GL in the intervention study. These observed effects were not confounded by gender, visit, age, height, weight, BMI, blood glucose and salivary cortisol values. Specifically, in the high GL groups participants reported feeling more 'confident', less 'sluggish', less 'hungry' and less 'thirsty', that is, they had improved mood before the CF tests. Similarly, in the low GI groups participants reported feeling more 'happy' and 'alert' (borderline), and less 'nervous' and 'thirsty' (borderline) (i.e. improved mood). Therefore, a low GI – high GL meal should be associated with the most improved mood, based on the results from this study. These results reported here are the first of its kind to reveal associations between the GI and GL of breakfast and mood.

Similar to the cross-sectional study, higher CHO was associated with feeling more 'tense', and less 'calm', which can be thought to represent a state of nervousness (arousal). The satiating effects of a high GL meal are not surprising, considering that the high GL meals had the highest energy content. There was in addition observed a GL and GI effect on how thirsty the children reported feeling. Since the liquid volume of the meals administered was the same between all meals, and the water consumed by the participants during the testing controlled (none of them had water after the meal, although they were allowed) it might in fact reflect a satiating effect. Indeed, hunger and thirst were shown to correlate highly ($r=0.7$, $p=0.001$). The satiating effects of a low GI *vs.* a high GI breakfast meal have already been documented, and it has also been suggested that changes in BG levels rather that the levels *per se* are strongly related to satiety (Buyken *et al.*, 2007); similarly, for high CHO meals (i.e. high GL) (Holt *et al.*, 1999). These findings suggesting that high GL and low GI meals are associated with decreased fatigue and increased alertness were similar between the two studies and in agreement with what has been suggested by Gibson and Green (Gibson & Green, 2002) (see section 2.1.3, page 33).

5.6 Potential mechanisms underlying the GI and GL effect on CF and mood

The intervention study in school children allowed for potential mechanisms to be considered, which could be underlying the GI and GL effect. In fact the potential mechanisms proposed here are in support of the Gibson hypothesis, where both the glucose and the cortisol levels, as a result of the meal administered and of the arousing situation (i.e. CF testing) interact to bring about effects on CF and mood (Gibson, 2007). This study is the first of its kind to investigate the effects of GI and GL on both glucose and cortisol levels after mixed meals in children, which have already been tested with regard to their glucose, insulin and cortisol responses.

The theory predicts that a high GI meal is going to enhance cortisol secretion under stress, while there should not be any differences between different meals in cortisol secretion when not under stress. "Under stress" is emphasized, because the responses are expected to be different under stress (study 3, in children) compared with no stress (study 2, in adults). Cortisol responses are highly variable even within individuals, and they depend greatly on the time of awaking. Therefore, with regard to both studies, in adults and children, the 'minus baseline' levels were considered, which allowed to take into account baseline variations, and thus reflect true changes in cortisol levels. When the 'minus baseline' levels were taken into account, in study 2 there were no differences under stress (i.e. study 2), while under stress the high GI meals were associated with increased cortisol responses (i.e. in study 3).

It could be argued that a high GI meal and, as a result of that, higher BG levels could result in stronger activation of the HPA-axis under demanding situations, which is reflected by the higher cortisol levels and participants reporting feeling more 'nervous', and thus better performance on vigilance tasks (i.e. how quickly information is being processed). On the contrary, a low GI meal, and as a result of that lower BG levels could result in lower activation of the HPA-axis under demanding situations, which is reflected by the lower cortisol levels and participants reporting feeling less 'nervous', and thus better performance on memory tasks. This proposed mechanism is not surprising considering that fasting, and as a result of that lower BG levels has been shown to result in a blunted HPA-axis response (Rohleder & Kirschbaum, 2007). Therefore, it could be argued that lower BG levels as a result

of a low GI meal could lead to a lower activation of the HPA-axis under demanding situations, which is confirmed in the present study. The opposite would be expected for a high GI meal, which is similarly confirmed by the findings. The differential effects of the cortisol responses on performance are again not surprising considering that glucocorticoids can cross the blood-brain barrier, and can influence memory and learning by binding to specific receptors; most importantly, that the differential affinity of Type I and Type II receptors could explain why higher cortisol levels could be associated with better vigilance (i.e. Type I activation), and lower cortisol levels with better memory when cortisol is exogenously administrated (i.e. Type II activation) (Lupien *et al.*, 2007). The endogenous effects of cortisol as a result of a meal and under a stressful condition have not been investigated. This is the first study to report findings in this area of research. In fact, the findings from Nabb and Benton (Nabb & Benton, 2006) could actually reflect this mechanism. Therefore, it could also be argued that it is not the effect of circulating BG levels on performance, but the effect of circulating BG levels as a result of the meal administered on cortisol levels after a challenging task, and subsequently on cognitive performance.

Performance on the tasks used in this study, contrary to mood, appeared to be particularly unaffected by the size of the CHO meal (i.e. GL). Only the Stroop task performance was related to GL. Therefore, it could be suggested that the observed GI effects were valid across the GL groups (low and high). In the introduction (see section 2.1.3, page 33), studies were reported that have shown an effect of the size of the meal on cognitive performance (Michaud *et al.*, 1991; Wyon *et al.*, 1997; Nabb & Benton, 2006). Nonetheless, the smaller size breakfast meals reported in these studies were very low energy breakfast meals (less than 10% of daily energy requirements, or 150kcal). In the present study, the low GL meals contributed 13% to the EAR (mean: 281.2kcal), and the high GL meals 21% to the EAR (mean: 470kcal). As such, it could be argued that the energy administered in the low GL meals in this study was not low enough for an effect on CF to be observed; and that perhaps, there exists a threshold effect (perhaps around 10% of the EAR) over and above which the size of the meal does not have an effect on performance 90 minutes later. The reason why performance on the Stroop task was affected by GL remains unclear, especially since it was not replicated for the other two vigilance tasks.

Research on the effects of glucose administration on cognitive function has shown that the glucose enhancing effects are evident not only during hypoglycaemia (Evans *et al.*, 2000), but also within normal glucose levels (Donohoe & Benton, 1999b; Hoyland *et al.*, 2008). Furthermore, recent research suggests that in addition to low blood glucose levels, the ability of a person to regulate BG levels within a normal range may affect performance (see section 2.2.2, page 42). The observation that after glucose administration higher pre-task BG levels (i.e. baseline and before the CF tests) and a greater drop in BG levels during the testing benefits performance has been based on the assumption that under 'mentally' demanding situations the brain is depleted of glucose. Therefore, those with higher levels of glucose (i.e. more glucose available in the circulation) perform tasks more efficiently. Nonetheless, the 'mental' demand is a term which is not consistently defined in the existent literature; rather, it has been suggested that an interaction exists between cortisol secretion and glucoregulatory processes, which seems to be mediating any observed effects – the Gibson assumption (Gibson, 2007). Furthermore, it has been recently suggested that changes in peripheral BG levels are unlikely to reflect changes of glucose supply in the working brain areas, as astrocytic glycogen is readily available under cognitive functioning to cover local demand (Messier, 2004; Brown & Ransom, 2007). In support of the latter is the fact that not only falling but also rising BG levels during testing have been associated with better performance (Benton & Owens, 1993; Owens & Benton, 1994).

Good glucoregulation has been described as the drop in glucose levels after glucose administration and during a challenging task, while poor glucoregulation as the state where BG levels remain raised reflecting a less efficient uptake of glucose from the circulation, and therefore result in poorer performance. Another important observation is that once the BG levels have dropped below baseline, the ability quickly to regain baseline values seems to be associated with improved performance (Donohoe & Benton, 2000); this could also be the effect of a the administration of a meal of specific composition (i.e. a low GI meal). It could also well be that different cognitive domains are differentially affected by blood glucose levels. Nabb and Benton (Nabb & Benton, 2006) demonstrated that only in people with better glucose tolerance (as assessed by fasting glucose levels <5mmol/L) lower levels of BG were related to greater word recall, while higher levels of BG were associated with quicker decision

times and more 'correct' responses on a vigilance task. In the present study, poor glucoregulation and hypoglycaemia were taken into account by excluding any participants that had either raised or low fasting BG levels. Therefore, any observed effects on BG levels should mainly reflect the interaction between meal administration, glucose levels and cortisol secretion during the CF testing. In conclusion, this study supports the assumption that circulating BG levels do not have an immediate effect on cognitive function on mood, but through their effects on cortisol levels result in CF and mood differences.

5.7 Limitations

5.7.1 Cross-sectional study

There are important limitations in the present study that need to be considered. First, the cross-sectional design means the observed associations may not be causal, leaving open the possibility that the relationships observed might be explained by unmeasured or residual confounders. What is more, the GI and GL values were based on what the participants reported having eaten for breakfast. The particular problem with the assessment of food intake in the present study is that we are dealing with adolescents. This presents several potential difficulties: the ability of children to keep and report diet accurately; the degree to which they are able to describe what and how much they eat, given the fact that they do not usually prepare foods; the biases in reporting, such as under-reporting and poor portion size estimates; the issue of motivation and boredom. For example, approximately 50% of the children in this age group under-report their intakes (NDNS, 2000). The use of calculated GI and GL meal values to predict glycaemic responses rather than actually measuring the glycaemic responses may compound the problem and help to explain some of the inconsistencies observed between the cross-sectional and the intervention study.

Although the low GI – low GL and the high GI – low GL meals had similar energy content, the calculated energy content of the low GI – high GL meal was 32.6% higher than that of the high GI – high GL breakfast. This difference was not statistically significant (Bonferroni post-hoc test). Nonetheless, the effect of the energy content *per se* cannot be strictly differentiated from the effect of the glycaemic

potency. A further extension of this problem relates to the macronutrient composition of the meals and its effect on both CF and mood. Again, it would have been desirable that within the same GL groups not only the energy content but also the macronutrient composition should be the same. (It would, of course, differ between the GL groups.) Post-Hoc Bonferroni tests showed that the difference in energy (as a percentage of EAR) was evident between the low GI – low GL and the low GI – high GL breakfast ($p=0.003$), and the percentage contributions of CHO and fat to energy differentiated the low GI – low GL from the high GI – high GL meal. The differences in composition, however, do not explain differences in CF as consistently as the observed combined effects of GL and GI in the two CF measures affected, the high GL and the low GI groups performing better (in accordance with the hypothesis); and of the effect of GL (i.e. high GL, better performance) or GI (i.e. high GI, better performance, not in accordance with the hypothesis) separately on two more measures.

Other limitations include the fact there was not available a baseline measure for either blood glucose or mood, which would have taken into account possible variations at baseline, and would have identified students with poorer or better glucoregulation. In the present study, time zero was based entirely on when the participants reported that their breakfast had started, and of course it was not the same for everyone, which may have introduced circadian differences as a variable which may have influenced performance. Additionally, the 30 minutes interval (i.e. 90-120 minutes after breakfast), which was allowed in order to test as many participants as possible, could have resulted in differences in glucose levels, cognition and mood, that were not measured. Thus, the entire testing procedure could not be timed precisely for all participating students. Furthermore, snacks that contained <10g of available CHO were not included in the total breakfast meal. Although these snacks were not included on the basis that they would not change BG levels, it should be noted that the introduction of other macronutrients into the stomach might alter the absorption profile of breakfast, thus altering BG levels and subsequent hormonal responses. In the cross-sectional study all snacks were CHO-rich and therefore included in the analysis. Although there were not any differences in the distribution of social class between the four GI and GL groups, the learning environment at home of the students,

which it was not possible to measure, may also have contributed to performance on the CF tests and hence on the differences observed (or not observed).

Finally, the GI values for the individual foods that constituted the composite meals were taken from the International Table of GI and GL values (Foster-Powell *et al.*, 2002); not all foods could be found in this table, and in some cases a value of a similar food in macronutrient composition had to be used. In addition, even when the exact food was found, there was variation in the reported values, or the foods tested were from different countries, which may also affect the GI values. Nonetheless, GI values for all foods and meals that might be recorded in observational studies are not currently available. Thus, selecting a GI value from the International Table and calculating GI in mixed meals was the only available approach in a retrospective assessment as in the cross-sectional study. It is likely that CHO and meal GI account for approximately 90% of the variation in glycaemic response, with non-significant effects from fat or protein (Wolever *et al.*, 2006). The use of the weighted GI/ GL in predicting the glycaemic responses in mixed meals has been validated by a number of studies (Wolever *et al.*, 1985; Wolever & Jenkins, 1986; Collier *et al.*, 1986; Chew *et al.*, 1988; Wolever & Bolognesi, 1996b; Wolever *et al.*, 2006; Galgani *et al.*, 2006). Specifically, the FAO/ WHO (FAO/WHO, 1998) accepts this prediction model, and states that 'the GI can be applied in a detailed fashion to mixed meals or whole diets by calculating the GI value of the meal or diet'. This was therefore the best that could be achieved for the purposes of the study, which was to generate hypotheses about the possible role of meal type on CF and mood.

Despite these limitations, the present cross-sectional study suggests that the GI and GL of breakfast may have affected performance in specific cognitive domains and (crucially) in real-life conditions. It also suggests that performance might be better after a low GI – high GL breakfast (i.e. two out of the four measures affected). The cross-sectional study is the first of its kind to consider both the GI and GL of breakfast when assessing the effects of breakfast on CF in school children, and to show that not only GI, but also the total CHO content of the meal together with its rate of absorption (as indicated by GL) are factors affecting cognitive outcome. It is also the first to report findings in early adolescents (as opposed to younger children or adults). The fact that performance in only four out of the seven tests administered was

associated with the differences in GI and/ or GL does not undermine the overall findings. It was never predicted that all of the CF tests would be equally affected. Indeed, theory predicts that differences in glucoregulatory processes and cortisol secretion under stress (i.e. arousal) may differentially affect performance in different cognitive domains after administration of meals differing in their GI and/ or GL. To what extent this is true was tested in the intervention study, although the full picture remains to be elucidated.

5.7.2 Predicting glycaemic and insulinaemic responses from mixed breakfast meals

The clinical study confirmed that both glycaemic and insulinaemic responses can be usefully predicted by both the CHO content and the GI of mixed breakfast meals, that is, the calculated GL. Furthermore, it provided the basis for the selection of the meals to be used in the intervention study in children. Indeed, a low GI – high GL meal, a high GI – high GL meal of similar macronutrient composition, a low GI – low GL and a high GI – high GL meal were selected. The most important finding is that during the 90 to 150 min interval that the CF tests were scheduled to be administered, both the GI and the GL of these meals predicted glucose and insulin levels. Therefore, there is no doubt that the meals selected differ in their glycaemic and insulinaemic responses; testing whether or not this is sufficient to produce differences in cognitive function and mood was the purpose of the final study.

The clinical study showed that the use of published GI values (Foster-Powell *et al.*, 2002; Henry *et al.*, 2005) to calculate the GI and GL values of the selected meals, and subsequently measure the glycaemic and insulinaemic responses, is an appropriate method. Despite the fact that when measuring glycaemic responses ideally all the foods selected should be GI tested, the clinical study showed that a careful selection of published GI values was sufficient to generate statistically significant GI and GL effects on glucose and insulin responses (at least for the foods used in the present study). Besides, measuring the GI of the individual foods of which the meals consisted, and then measuring the glycaemic, insulinaemic, and cortisol responses of the meals would have been impractical in the time available.

The study also confirmed that the use of capillary blood to measure the glycaemic responses is the preferred method, as it produced more highly statistically significant correlations, compared with the venous blood (FAO/WHO, 1998). One important limitation is that a portable BG meter was used to measure the capillary whole blood glucose, rather than collecting the drops of capillary blood in tubes and analyzing it. Nonetheless, the meters selected have been validated for extra-laboratory use, and are the only available method to measure blood glucose in epidemiological settings (i.e. schools). Therefore, the use of a clinically acceptable glucose meter should not significantly undermine the associations between the observed glycaemic responses and the predicted GI and GL values.

Finally, the clinical study is the first of its kind to show that cortisol responses are unrelated to GI and/ or GL, and that there are no differences in glycaemic, insulinaemic and cortisol responses between males and females. The fact that higher GI of the meal consumed on the evening before the testing was associated with higher glycaemic and insulinaemic responses the following morning confirms previous findings (Wolever *et al.*, 1988; Granfeldt *et al.*, 2006), and highlights the importance of standardizing or controlling for the evening meal in such studies, wherever possible.

5.7.3 Intervention study in children

The main advantage of the intervention study is that it measures exposure (i.e. GI and GL of breakfast – dietary manipulations) and outcome (i.e. cognitive function, mood) under more controlled conditions compared with the cross-sectional study; and that it can draw conclusions as to whether the intervention has effects under day-to-day conditions (i.e. apply to the 'real world').

There are limitations in this study that need to be considered. First of all, the design of the study: participants were exposed to only two out of the four test meals. Each subject was allocated to either a high or a low GL group, and received the low and high GI meal within the same GL group. Although participants were matched between the low and the high GL group, this can not be considered as a repeat measures design where all participants are exposed to all experimental conditions,

which could be regarded as a stronger design. This is why GI was a within-subject factor and GL a between-subject factor. Nonetheless, exposing the students to all four test meals would pose different limitations, potentially more damaging to the integrity of the study. Doubling the number of the visits (i.e. five in total with the screening) would increase the drop out rates, which were high in any case. In addition, it would increase both the observer effects and the participant effects. The blindness of the observer was difficult to control in this study, since the meals administered could be easily seen, (although they were not prepared by the same observer who made the CF measurements). Furthermore, the intra-observer differences were controlled by having the same observer administer the tests for the same child, and the inter-observer differences by thoroughly training the participants and selecting time of administration as a standardized comparison of the differences between observers. There was not a point at which any one observer collected all the information for a given test within a GI or GL group. Therefore, any differences observed can not be attributed to between-observer differences. The participant effects effectively represent expectancy effects and familiarity with the tasks (and the meals), which may result in ceiling effects or even loss of interest.

Furthermore, there are two points that need to be addressed with regard to the intervention study, and the cross-sectional one. First, in the cross-sectional study the 1990 Government Statistical service (SOC90) was used to derive the socio-economic groups, while in the intervention study the 2000 Government Statistical service (SOC2000) to derive analytic classes (i.e. social class based on occupation), which is a revision of the former SOC90. This discrepancy between the two studies is not likely to be critical, as SOC90 and SOC2000 are closely comparable and were used to characterize social-class within the same group of subjects (i.e. within a given study). Second, in the cross-sectional study, Microdiet was used for the nutrient analysis, and in the intervention study the FSA Nutrient Databank. Again, this should not have had a material impact on the calculation of GI and GL or the outcome. There is a enormous amount of commonality between the Nutrient Databank and McCance and Widdowson's food composition tables, on which the database in Microdiet is based.

The fact that a baseline measure of performance (i.e. before meal administration and ideally on a day where participants would consume their normal breakfast) was not available could be considered as an important limitation. Nonetheless, in studies of this kind, a baseline measure of performance was usually not acquired (Wesnes *et al.*, 2003; Ingwersen *et al.*, 2007), and it would probably complicate findings, as the interest is in short-term differences of high or low GI meals on cognitive function, and not on whether there is an improvement or decline in overall cognitive function as a result of a meal. The latter would be addressed as the outcome of a long-term trial.

Another limitation that needs to be considered in studies of this kind is the speed-accuracy trade-off. This effectively means that participants on some tasks might slow performance and maintain accuracy, and on others maintain speed at the expense of accuracy. The speed-accuracy trade-off effect is difficult to control for, and the least that can be done to prevent it is to provide participants with specific directions, for example 'please do this as quickly and as accurately as possible' (e.g. as it was done for all the vigilance tasks: Stroop task, speed of information processing and serial sevens).

Finally, one further theoretical limitation is the order of administration of the CF tests themselves. The tests were administered in the same order for every participant and on both occasions, rather than the order being randomized. It could be argued that by administering the tests in the same order, there may be an interaction between the tests which might have endangered or obscured an effect of GI or GL. It was thought, however, that the likelihood of interactions between tests (and hence any advantages of randomization) was small, and that the possible benefits of randomization would be outweighed by possible disadvantages, principally the complex logistics associated with creating different versions of the test administration booklet for each child which then raised the risk of loss of adherence to the test protocol.

5.8 Public Health Implications

5.8.1 Overall conclusions

This research is the first of its kind to consider both the GI and GL of breakfast when assessing the effects of breakfast on CF in school children, and to measure glucose and cortisol levels. It is also the first to report findings in early adolescence. An important overall advantage is that the meals administered had already been tested with regard to their glycaemic, insulinaemic and cortisol responses under no-stressful conditions, which similarly has never been conducted by any of the investigative groups in this field.

In conclusion, it appears at first glance that the findings from the PhD studies are not in support of the hypothesis that a low GI – high GL breakfast is beneficial to school children. Nonetheless, a closer look reveals that the findings are in fact in support of the hypothesis. First of all, it appears that the GI effect is domain specific; that is, low GI meals across GL groups appear to be selectively facilitating memory performance, and high GI meals across GL groups to be selectively facilitating vigilance (i.e. how quickly information is being processed). In a school environment, the ability to retain newly acquired information and to access and recall/ retrieve information already stored in memory is more important for learning, compared with how quickly information is processed. Therefore, a low GI meal would be more likely to be beneficial to school children, especially since it is also associated with improved mood. Participants having received the low GI meals reported feeling more 'happy' and 'alert' and less 'nervous' and 'thirsty'. Besides, based on purely physiological grounds, a high GI meal was shown to be associated with higher insulin levels and higher cortisol levels under stress, which in the long-term is unlikely to be advantageous to health. As far as GL is concerned, a high GL meal would be the preferred one as it resulted in participants feeling more 'confident', and less 'sluggish', 'hungry' and 'thirsty' compared with the low GL. This effectively means that it resulted in improved mood and more satiating effects, which could be implicated in better behaviour, and subsequently better performance. Moreover, based again on physiological grounds, only the low GI – high GL breakfast preserved glucose levels above baseline for the entire testing period, that is three hours,

preventing levels below baseline and/or hypoglycaemia, which could potentially have detrimental effects on performance (Evans *et al.*, 2000) (see Figure 4.4, page 157).

These novel findings shed new light in this area of research, and have helped to provide a clearer idea of the potential underlying mechanism. These findings remain to be confirmed not only in further short-term studies, but in long-term intervention trials as well.

5.8.2 Generalizability of findings

The issue of generalizability (i.e. external validity) refers to whether there is some biological difference between the studied population and the general population. Usually it is assumed that the results are generalizable, unless there is strong reason to suspect otherwise. The findings of the current research are physiologically and psychologically representative. While the current study is not a random sample of the entire UK school population, it is a population-based sample of adolescent school children from five different schools with a wide variety of abilities and mix. Furthermore, there is no strong reason to suspect that the biological effects of the glycaemic potency of breakfast in these adolescent school children will be different than adolescent school children in general. Hence, the present PhD provides an opportunity for efficient use of high-quality data from a large, well-described, population-based intervention trial to yield highly informative findings regarding the effects of the glycaemic potency of breakfast on cognitive function and mood in adolescent school children. The low response rate (<15%) could be an issue of internal validity (i.e. selection bias). Nonetheless, the potential selection biases are hard to overcome, since the many ways that respondents differ from non-respondents can not be known.

The current research was able to demonstrate an impact of the nutrient different breakfast meals on mood and cognition in school-aged children. The findings reported in this thesis are representative of the general school population aged 11-14 years; they are not representative of younger or older children. First of all, the schools were selected on the basis that they were mixed comprehensives, with a wide social class and ability mix. Furthermore, the sample of the children that took part in the studies

were not much different from the general population, as the mean height of boys and girls aged 13 years in the recent NDNS data is 157 (sd=9) and 158 (sd=7.2) respectively, which is similar to the ones reported in this thesis (156 (se=1.4) and 158 (se=1.1), respectively). The mean weight of boys and girls 13 years in the recent NDNS data is 48 (sd=9.4) and 53 (sd=13.1) respectively, which again similar to the ones reported in this thesis (46 (se=1.2) and 51 (se=1.3), respectively). Therefore, there is no reason to believe that the findings reported are not physiologically and neurologically (i.e. biologically) representative of the general population.

5.8.3 Relevance to public health nutrition

These novel findings could influence government education policies and public health attitudes to breakfast. Besides, the purpose of the current research was not simply to clarify the associations between the glycaemic potency of breakfast, cognition and mood, but ultimately to contribute to the promotion of the education of children and young people through consumption of better quality food. The observed effects of GI/ GL on mood and cognition of school children suggest that providing a breakfast with an improved nutrient profile could in fact increase the learning potential of children. Nonetheless, for such an effect to be realized, the following strategy would have to take place: parents, children and food providers would need to be educated and appropriately guided into being able to recognize and select breakfast meals with enhanced nutrient profiles; food providers and cooks would need to use foods/ products in food production and preparation that meet the recommendations; and local and national agencies would have to aid these efforts with policy and legislation that promotes breakfasts with specific nutrient profiles. An extension to the latter would be for the governments to provide free breakfast meals with specific nutrient profiles to all children at school, which could in theory lead to improved academic performance. Other recent findings suggest that better behavior and even reduced truancy rates could result from better eating at school. The educational approach should be developed in such a way to establish good food not only as part of the school day, but also to ensure that the healthy eating habits developed by children at school are integrated into their everyday lives. Providing breakfast meals with low GI – high GL would be likely to result in academic benefits including – but not limited to - enhanced cognition, and in consequence improved grades.

Currently there are a lot of gaps in the evidence base to allow for firm recommendations to be made. This can be attributed to the inherent difficulties in understanding the complexity of the many factors that have an impact on school children's eating habits. Yet it is reasonable to assume that over the 12 years of a child's school career there will be important impacts of both food consumption and learning about food within the school environment that will have both short-term and long-term consequences. More crucial still, the school environment provides unparalleled opportunities for (a) practical interventions relating to food and health that can be integrated into the curriculum and wider learning experiences and (b) follow up in young and middle adulthood. Equally, the impact of factors outside of school may undermine school-based interventions, and the wider context of eating in childhood (especially in adolescence) needs to be addressed.

This PhD research is the first of its kind to provide robust research to suggest that better breakfasts may be associated with better cognitive function and hence better academic outcomes for children and young people. Ultimately, of course, consumption will be determined by an individual child's likes and dislikes (including aspects of the eating environment both at home and at school). These likes and dislikes evolve over many years, and the ability to influence diet in the school years will in part be determined by pre-school factors, from breast-feeding onwards. Therefore, there is a need for implementation of any policies regarding school food at an early age. The supporting or opposing influences on a child's food choices in school and outside of school need to be recognized (i.e. any influences through the media (i.e. Jamie Oliver's shows in the UK)), even if they cannot always be taken fully into account in terms of research design.

The importance of breakfast vs. no breakfast in school life and performance has been long established. The present research further demonstrated that the macronutrient composition of breakfast is not only of importance, but can also lead to improved mood and cognition. Of course, long term intervention trials are needed in order to establish whether the long-term consumption of a breakfast with a specifically designed composition can enhance academic performance. Therefore, the following two studies are proposed in the next section (a) to confirm the findings from the present research and (b) to establish associations under chronic exposure.

5.9 Recommendations for future studies

Several recommendations can be made to improve the design and methodology in studies investigating the effects of varying the GI and GL (or even the macronutrient composition) of breakfast on performance.

- ❖ Employ a within-subject double blind design, but not at the expense of other factors (e.g. familiarity effects), and have a baseline measure of performance
- ❖ Employ a fully balanced design, based on the exposure variables of interest
- ❖ Both the GI and GL should be taken into account when assessing the effects of the blood glucose raising potential of the meals on performance and mood
- ❖ The macronutrient composition of the meals should be kept the same, and only the CHO source should vary, if GI effects are to be investigated
- ❖ The differences between low and high GL meals should be at least two-fold to allow for significant changes in glucose levels to be detected
- ❖ Measure the glycaemic responses of the meals to be tested, and determine appropriate time to test for peak effects on performance (or evaluate performance at several time-points)
- ❖ Control/ account for length of fast prior to experiment, and other variables (e.g. exercise, caffeine, hours of sleep, dinner the evening before etc)
- ❖ Consider effects of mood, hunger, motivation, task demand, and fatigue
- ❖ Assess the complexity of the tasks, and vary mental demand (though a unified approach has not been employed so far)
- ❖ Use a wide range of CF tests, even within the same cognitive domain to establish task sensitivity
- ❖ Account for unfamiliarity with the tasks (i.e. practice/ introductory session), and for practice effects (i.e. determine baseline levels of performance at the breakfast habitually consumed by the participants)
- ❖ Employ a no breakfast condition to be able to elucidate more accurately the underlying mechanisms (especially with regard to glucose and cortisol levels and their effects on performance)
- ❖ Time the procedure accurately, by measuring all participants at the same time of the day (to avoid circadian effects on performance)
- ❖ Determine glucoregulatory status of participants (e.g. baseline glucose levels, glycosylated haemoglobin, glucose tolerance test)

❖ Measure blood glucose and cortisol levels at baseline, and before and after the tests

Overall, this field of research lacks a unified approach and standardized methodology and terminology, not only with regard to the sensitivity of measures, but in addition to determining and accounting for possible confounding factors. This, to me, should be the primary aim of future studies in this field.

With regard to future studies in this field, two intervention studies might be proposed. The first would be a study in which the short-term effects of the glycaemic potency of breakfast on cognitive function would be replicated, especially with regard to the memory effects, time effects and mood. The second would be a study in which the long-term effects of administering a low GI – high GL breakfast would be investigated not only on cognitive function and mood, but also on academic performance.

The hypothesis of the first study would be that a low GI breakfast selectively enhances memory performance, while a high GI breakfast selectively enhances vigilance in school children. The GL would not be expected to have an effect except in relation to mood. Tests assessing only memory and vigilance would be administered, with a particular emphasis on selecting sensitive memory tasks (i.e. declarative verbal memory). Computer based tests would be used rather than paper based, which would allow for more subjects to be tested on a single day. In order to replicate the findings with regard to GL (i.e. no effect on performance) all participants would be exposed to all experimental conditions. Therefore, a baseline measure of performance would have to be established. Although the PhD research clearly demonstrated that a low GI – high GL breakfast best facilitates mood, mood would also have to be assessed in order to take any changes in mood into account at a given level of GL (even if GI were the target of variation). This study could also be repeated at different time-points (not only 90 min after breakfast), in order to investigate whether the GI effects hold true for earlier or later time-points.

Of course, the implications of identifying the macronutrient composition of breakfast that could best facilitate cognitive performance after an overnight fast would be to investigate whether a particular breakfast, e.g. low GI – high GL breakfast, has similar facilitating effects on academic performance and behaviour. The hypothesis of a long-term intervention trial would therefore be that consistent consumption of low GI – high GL breakfast improves academic performance and behaviour over a period of 3-6 months. Measures of academic performance would include school grades based on standardized tests, while measures of behaviour would include attendance and truancy records, direct observations of behaviour in the classroom, and mood. The cognitive function tests would have to be adapted for the six month interval. The interest would be on whether breakfast alone can have an effect on academic performance. Anything eaten during the school day (i.e. snacks, lunch) would be recorded and controlled for (confounding factors) at key time points throughout the period of intervention. The interest would be on whether breakfast can influence academic performance and behaviour at school, and not on whether changing their diet as a whole would have an effect. The overall diet would need to be assessed (and possibly controlled for, but this would substantially complicate the protocol) using either food frequency questionnaires or four repeat 24 hour dietary recalls at baseline, and then again every month. Techniques for assessing total diet would need to be assessed with the involvement of parents/carers and the children, to minimize misreporting.

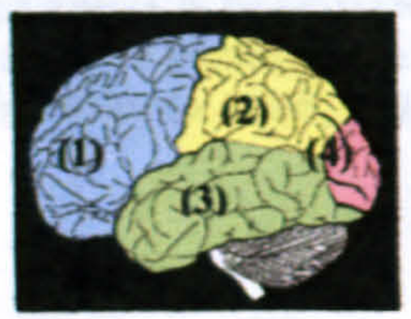
APPENDICES

I. Literature review

I.1 Structure and Functions of the brain

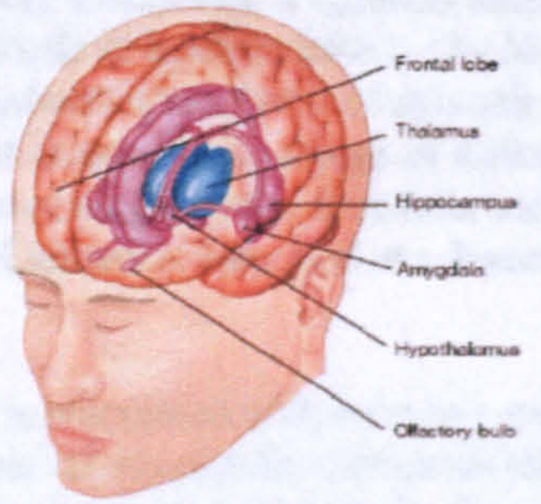
The human brain can be divided into three main regions that have different functions: the cerebrum, the cerebellum, and the brain stem. The part of the brain involved in higher executive functions, such as reasoning, is the cerebrum, and therefore is going to be the main focus. The cerebellum's functions are carried out subconsciously, and involve processing of sensory information and coordination/ execution of movement. The brain stem is an extension of the spinal cord, and contains centers for many involuntary actions, such as eye movement, coordination of breathing, arousal, sleep, muscle tone, pain modulation, blood pressure regulation. The cerebrum is the largest part of the brain and is consisted of the two hemispheres (right, left) and the diencephalons (thalamus – relay station of sensory information from lower parts on its way to the cerebral cortex, and hypothalamus – homeostasis, hunger and thirst, emotion and motivation, stress reactions). Each hemisphere is divided into four lobes, frontal, parietal, temporal, and occipital. These lobes control different functions:

Frontal lobe (1): Concentration; problem solving; behavioural control and temperament; motor association areas; coordinates messages from other lobes.
Parietal lobe (2): Sensory association area (from skin, musculoskeletal system, viscera, taste buds).
Temporal lobe (3): Auditory association (i.e. hearing); taste, smell.
Occipital lobe (4): Visual association (i.e. vision).



So, far the discrete areas of the brain have been mentioned. Nonetheless, some of the parts of the cerebrum form together an operational system, the limbic system (Figure 1). The structures of the limbic system are related to learning, memory, emotion and behaviour. The amygdala and the hippocampus are part of the interior of the cerebrum, and are linked to emotion and memory, and learning and memory, respectively (Silverthorn, 2001).

Figure 1: The limbic system; linked to learning, memory, emotion and behaviour.



Source: Silverthorn (Silverthorn, 2001).

The frontal lobe is a part of the limbic system. Therefore, it would be worth becoming familiar with the executive functions mediated by this part of the brain. An extensive review has been published elsewhere (Stuss & Levine, 2002); as such, the main points are going to be briefly mentioned.

The frontal lobe can be divided into two main neuroanatomically distinct parts: the ventral prefrontal cortex (VPFC) – affective functions, and the dorsolateral prefrontal cortex (DLPFC) – cognitive functions. The DLPFC originates in the hippocampus, and is implicated in spatial and conceptual reasoning processes (i.e. executive processes). The VPFC emerges from the olfactory cortex,

and is involved emotional processing and behavioural self-regulation (i.e. inhibition, emotion).

Cognitive functions

The cognitive functions associated with the prefrontal cortex (dorsolateral) include higher level language, memory processes and attention. Though functionally discrete, the operations involved in the control of these functions are interlinked. The tests used to assess these functions are called 'frontal-tests'. The most popular frontal-test is the letter-based fluency task (word generation) where participants have to generate a list of words beginning with a certain letter, which traditionally reflects left frontal function (Stuss & Levine, 2002).

Memory is a major cognitive process that allows the storage of learned information, and the retrieval of these information when required. This complex process can be classified in different ways: based on the duration of the retention to short- and long-term memory; based on the type of content to auditory or visual, spatial or verbal etc; according to the type of cognitive process to non-declarative (implicit or procedural) and declarative (explicit) memory; and to strategic and non-strategic. 'Non-declarative memory deals with learned or conditioned responses, such as habits and sensorimotor skills (i.e. procedural memory of action chains)'. Declarative memory represents the verbal retrieval of information, readily available to the conscious recollection (i.e. can be declared), that can be either semantic or episodic. Episodic memory involves an association of particular set of events, which describe a past episode, reflecting autobiographical memory, and semantic memory reflects factual information about the world, including vocabulary items and knowledge about what certain items are used for. Finally, strategic memory is a higher executive function that involves the use of many cognitive processes to be performed (e.g. writing a PhD thesis!), while non-strategic memory passively educes response to external stimuli (Westenhoefer *et al.*, 2004).

Memory is controlled by both the medial temporal lobe/ hippocampus and the frontal lobes. The former are involved in basic associative processes of cue-engram interaction, and the latter in strategic processes involved in the coordination, elaboration, and interpretation of these associations; mainly control and direction. Specifically, the hippocampus is involved in spatial tasks (special cue tasks with objects or particular places), in which a memory of a particular episode or context is required, rather than that of a general rule (non-spatial tasks); effectively it controls associations between objects and places (Rolls & Kesner, 2006). The hippocampus is involved in the formation and retrieval of episodic memory using auto-association, that is associating things of different context, places, or people as one single event. Semantic memory is controlled by mainly the medial temporal lobe, and at a lesser extent by the hippocampus.

The left frontal lobe is associated with memory encoding, and right frontal lobe with retrieval of episodic memories (Stuss & Levine, 2002). Mnemonic processes mediated by the frontal lobes include subjective organization (pair-frequency), encoding and retrieval; particularly episodic memory retrieval (long-term memory). Working memory (part of declarative long-term memory) is another process mediated by frontal lobe functions; including encoding strategies, storage/maintenance, rehearsal, interference control and inhibition. These processes require some level of attentional control.

Digit span or spatial span tasks are important for determining working memory storage capacity, while digits backwards in addition measures manipulation of information held on-line. Other tests assessing working memory tasks involve the Wechsler Instruments (manipulation and control); the Brown-Peterson technique taps (in the presence of interference); and supraspan tests (processing when working memory capacity is exceeded). Delayed response tasks including self-ordered pointing and conditional associative learning tests, assess other mnemonic processes of

frontal lobe functioning. The self-ordered pointing task requires the participant to avoid repetition by monitoring past responses. The conditional associative learning tests use trial and error technique to acquire associations between a set of stimuli and a set of responses (Stuss & Levine, 2002).

The **attention** processes controlled by the frontal lobes can be divided into attentional switching (divided attention), selective attention, and sustained attention. Attentional switching involves 'generation and identification of concepts, hypothesis testing, maintenance of attention, resistance to interference, utilization of feedback to guide behaviour, and when more than one concept is possible, switching categories and inhibiting perseveration of prior categories'. Tests used to assess attentional switching involve the Wisconsin Card Sorting Test (WCST), the Cambridge Neuropsychological Test Automated Battery (CANTAB), and the California Card Sorting Test (CCST), and the Trail Making Test. Selective attention involves omitting responses to important stimuli or reacting to irrelevant information; the Stroop task is the most predominantly employed test. Sustained attention, which involves 'detection of targets over a prolonged period of time', lacks widely accepted measures. Among the ones used are letter cancellation or other 'vigilance' tasks, continuous performance tests (repetitive tasks maintaining endogenous arousal) (Stuss & Levine, 2002).

Overall, cognition represents a multidimensional set of abilities, and despite classification into separate domains, the various cognitive processes mentioned before are interlinked. The various CF tests used are composite, and they can not specifically disentangle which frontal regions are involved. Generally, the more complex the function the more parts of the frontal lobe are involved.

Emotions and behaviour

The control of emotions and behaviour, such as decision making, which is mainly mediated by VPFC and the frontal cortex, is beyond the score of this report. Amygdala is also involved in the regulation

of emotions, and in the emotional leaning and memory. Specifically, the amygdala 'subserves incentive learning, the process through which sensory-perceptual features of appetitive (or aversive, i.e. Pavlovian fear conditioning), and rewarding (or punishing) events acquire affective significance and, hence, the ability to motivate or incite responses and actions' (Balleine & Killcross, 2006). Briefly, the operations regulated are high level decision making; acquisition and reversal of stimulus-reward associations; reversal learning (i.e. affective); regulation of behaviour according to inner goals and limitations; retrieval of episodic memory (personally/ emotionally related to the individual); self-reflective memory; empathy, sympathy, and humour (i.e. self-awareness).

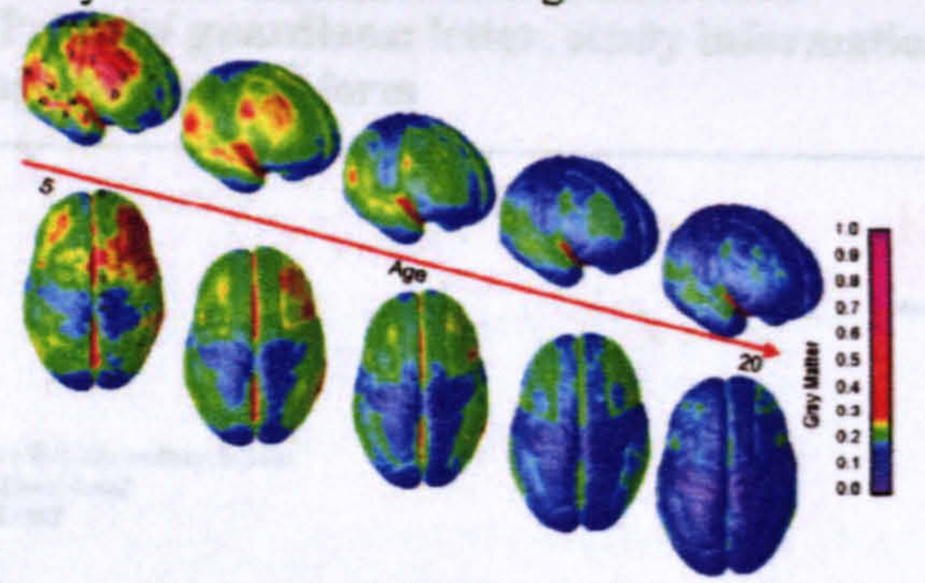
I.2 Adolescent Brain

Adolescence is a critical period in the life of an individual, where emotional and cognitive changes take place, imperative for independent functioning. It is very difficult to put adolescence into chronological context, as there is no single event that indicates its onset or termination. Adolescence is usually placed between the ages of 11 to 18, without excluding the possibility to exceed to the early twenties. The physical growth observed in adolescence, is generally related to growth in cognitive abilities (Spear, 2000). During adolescence a maturation of neurological processes takes place – social and emotional behaviour and cognitive abilities. Magnetic Resonance Imaging (MRI) techniques have shown the structures and functions of the brain that are involved in this maturation (Yurgelun-Todd, 2007). Although there is little increase in brain size after the age of five years, during adolescence there is observed a major transformation of cognitive thought leading to age-related improvements in higher executive functions, such as abstract reasoning.

The developmental plasticity the brain undergoes during adolescence is reflected by the observed linear increases in white matter (WM) and decreases in cortical grey matter (GM) (Gogtay *et al.*, 2004); specifically, gray matter increases during pre-adolescence, peaks early in the frontal cortex during adolescence, but then decreases during post-adolescence. Figure 2 depicts the loss of GM during adolescence and early adulthood. The increases in WM may reflect increases in myelination, and the decrease in GM the pruning of synapses in the cerebral cortex of the adolescents, both of which are associated with age-related improvements in cognitive functioning and control of impulse (Yurgelun-Todd, 2007). This synaptic pruning is related to an increase in the focal rather widespread activation of the brain, and a subsequent decrease in brain activity, as estimated from rates of glucose metabolism, oxygen utilization, and blood flow (Spear, 2000). Due to this developmental plasticity it could be argued that it is a crucial period in the life of an individual, where he/ should be exposed to a rich environment of both mental stimulation and nutritional adequacy.

The maturation of the prefrontal cortex is accompanied by an increase in higher executive abilities, including abstract reasoning, attentional shifting, decision making, response inhibition and speed processing, and emotional control – affective inhibition (Yurgelun-Todd, 2007). Gogtay et al (2004) showed that the non-linear development of GM from childhood to early adulthood is accompanied by a relevant improvement and maturation in cognitive abilities. Therefore, parts of the brain associated with more primary functions (e.g., primary motor cortex, frontal pole, occipital pole: motor and sensory areas) appeared to develop earlier; followed by areas involved in spatial orientation, speech and language development, and attention (upper and lower parietal lobes); last to mature were areas involved in executive function, attention, and motor coordination (prefrontal cortex, temporal cortex).

Figure 2: Grey matter maturation during adolescence.



Source: Gogtay (Gogtay *et al.*, 2004). 'Right lateral and top views of the dynamic sequence of GM maturation over the cortical surface. The side bar shows a colour representation in units of GM volume'.

It seems that the widespread belief that cognitive abilities 'spurt' during adolescence should be interpreted with caution. Someone would expect, based on the what was previously reported, for some cognitive abilities to increase during childhood and then to level-off during adolescence, and for others to increase linearly from childhood to adulthood. Recent cross-sectional results support this assumption (Waber *et al.*, 2007). Higher executive functions, such as coding matrix reasoning, block design, spatial working memory and passage comprehension were improved in adolescence, while others such as verbal fluency and verbal learning, adult levels of performance were approached between the ages of six to ten.

II Cross-sectional study in school children

II.1 Letter to the Headteacher

School of Health and Life Sciences
Department of Nutrition & Dietetics

Franklin Wilkins Building
150 Stamford Street
London SE1 9NH
Tel 020 7848 4268
Fax 020 7848 4185
Direct line 020 7848 4349
Email: michael.nelson@kcl.ac.uk

30 March, 2005

Mr C Garvey
Headteacher
Sacred Heart School
Camberwell New Road,
London, SE5 0RP

Dear Mr Garvey,

Learning ability in teenagers

We have recently carried out research to investigate relationships between children's iron status and their cognitive function. We have shown in both boys and girls that iron deficiency has a significant detrimental effect on cognitive performance, independent of social class, age and ethnic background.

I am writing to ask if you would be willing for pupils in your school to take part in a new study. For many years, there has been controversy regarding the role of breakfast eating on cognitive function in children. We believe that these studies have been flawed for a number of reasons, and that the relationship between breakfast eating and cognitive function depends not only on consumption of breakfast *per se*, but also on the type of breakfast, insulin response and glucose levels, iron status and mood. Please find enclosed a brief summary of the research; a draft letter, questionnaire and consent form for parents; and a draft consent form and Information Sheet for participants. This research has been approved by the Research Ethics Committee of King's College London (REC protocol number: 04/05-51).

I appreciate that teachers face a heavy workload. We have designed the study to avoid any additional workload on teachers. For those pupils willing to take part, we would need about one hour of their time. The work would be carried out by Renata Micha, a PhD student working under my supervision.

I am sure that you believe that we must do all that we can to help children develop and express their full potential at school. I hope that you feel able to support this request for research at Sacred Heart School. Thank you for taking the time to consider this request.

Yours sincerely,

Dr Michael Nelson
Reader in Public Health Nutrition

[1]The findings were published in the *Proceedings of the Nutrition Society*: Williamson CS, Al-Hamad H and Nelson M. Iron status, diet and cognitive ability in schoolboys aged 11-14 years. 2002, 61, 56A.

[2]Nelson M, Ash R, Mulvihill C, Peters TJ, Rugers P. Iron status, diet and cognitive function in British adolescent girls. *Proceedings of the Nutrition Society* 2001;60:59A

II.2 Parents/ guardians: letter, study information sheet, screening and consent form

Sacred Heart R.C. Secondary School
Camberwell New Road
London SE5 0RP

9/May/2005,

Dear Parent/Guardian,

Breakfast eating and learning ability in adolescents.

Sacred Heart R.C. Secondary School is helping the Department of Nutrition and Dietetics at King's College London with research on how the type of breakfast eaten by adolescents may affect how well they do at school.

Recent research suggests that breakfast consumption improves school attendance and academic performance at school. The results are not consistent, however, because a number of important factors, including the type of breakfast, iron status and mood have not been taken into account.

It is very important that we learn more about how the type of breakfast that adolescents eat may affect their learning ability. Each child who takes part in the study will be asked to complete a short questionnaire, tests of learning ability and a mood scale. We also need to take a finger prick blood sample to see how the levels of iron and glucose in the blood affect performance on the tests of learning ability. We will be able to tell you if your child's iron levels are too low. We will write individually to the parents of all students whose blood tests suggest that they have poor iron status.

Please find enclosed an information sheet, which explains the research in more detail. We also enclose a separate information sheet for your child.

When you have read the information sheet overleaf, we would be grateful if you would complete the enclosed consent form and screening questionnaire and return it to the school in the envelope provided. Co-operation is of course voluntary, and we will be grateful for any assistance that you and your child can give us.

Yours sincerely

Ms Sally Coates
Headteacher of Sacred Heart
R.C. Secondary School

Dr. Michael Nelson
Reader in Public Health Nutrition
King's College London

INFORMATION SHEET FOR PARENTS

Sacred Heart R.C. Secondary School
Camberwell New Road
London SE5 0RP

BREAKFAST EATING AND LEARNING ABILITY IN ADOLESCENTS

We would like to invite your child to participate in this research project looking at breakfast consumption and learning ability. Before you decide whether you want your child to take part, it is important for you to understand why the research is being done and what your child's participation will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. If you do decide for your child to take part, please let us know beforehand if he/she has been involved in any other study during the last year.

Why are we carrying out the research?
Recent research in school-aged children shows that breakfast improves school attendance and academic performance. However, the results are not consistent. Positive effects of breakfast consumption on learning ability may depend on the type of the breakfast rather than the consumption of the breakfast itself. We want to know if particular types of breakfast are related to better performance in tests of learning ability. We also want to know if mood is affected by eating breakfast, and if it has an effect on the tests of learning ability.

Who will take part in the study?
All children aged 11-14 in the Sacred Heart R.C. Secondary School will be asked to take part. Not all children who consent will be participating, but some who will be selected at random. Children who have any of the following illnesses will not be selected: anaemia, thalassaemia, sickle cell anaemia, other causes of anaemia (hookworm infection, schistostomiasis and malaria), diabetes or other disorders of glucose metabolism, or other acute or chronic illnesses or diseases. The school was selected because the children who attend come from a wide variety of backgrounds. Children can take part when they and their parents or guardians have signed a consent form saying that they understand what the study involves. Any child who starts the study but then changes his/her mind is free to stop at any time without having to give a reason.

What will each child be asked to do?
All children who agree to take part in the study will be measured for height and weight, and will be interviewed individually by either Miss Renata Micha, Miss Julia Forbes, or Miss Kathryn Lowes. The interview will include questions about your child's eating habits (how often he/she eats breakfast, if he/she is a vegetarian etc), his/her physical activity, and his/her current health status. Each child will be asked to complete tests of learning ability (for example tests of memory and concentration) and questions about mood (for example if the child is tired, hungry, happy etc). We will take two finger prick blood samples, one before and one immediately after completing the tests and mood scales. We will measure haemoglobin (measure of iron status), and blood glucose levels. We want to measure these because iron status and blood glucose levels are associated with learning ability. There is no risk of infection when the finger prick blood samples are taken, as there will only be used sterilized equipment and disposable lancets. The whole procedure will take approximately one hour, and it will take place during lesson time. We will write to you if your child's blood tests suggest that he/she has poor iron status.

This study has been approved by the King's College Research Ethics Committee, reference 04/05-01

INFORMATION SHEET FOR PARENTS

Who will see this information?
Only the people from King's College London who are directly involved in the research will see the information for individual children. The information collected will not be shown to anyone else. All the information collected will be kept strictly confidential. No individual child will be identifiable in any verbal or written reports about the research. At the end of the study, each child and his/her parents or guardians will be told about the results of the learning ability tests and whether the type of breakfast affects the students' performance on these tests. Parents will also be informed if their child has low iron levels.

When will the work be carried out?
The study will be carried out between May and June 2005 by research assistants at King's College London. If you have any questions about the study, please ring 020 7848 4349.

It is up to you to decide whether or not your child should take part. If you do decide for your child to take part you will be given the information sheet to keep and be asked to sign a consent form. You may freely withdraw your child at any time and without giving a reason, even when parental consent has already been given. In the event of your child suffering any adverse effects as a consequence of his/her participation in this study, you will be compensated through King's College London's 'No Fault Compensation Scheme'.

For further information please contact:
Dr Michael Nelson
Reader in Public Health Nutrition
Department of Nutrition and Dietetics
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Franklin-Wilkins Building
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London SE1 9NH
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e-mail: michael.nelson@kcl.ac.uk

Renata Micha
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Department of Nutrition and Dietetics
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e-mail: emi.micha@kcl.ac.uk

This study has been approved by the King's College Research Ethics Committee, reference 04/05-01

2

PARENT OR GUARDIAN SCREENING AND CONSTENT FORM

Sacred Heart R.C. Secondary School
Camberwell New Road
London SE5 0RP

BREAKFAST EATING AND LEARNING ABILITY IN ADOLESCENTS

I have read and understood the study information sheet overleaf.

I understand that I may freely withdraw my child from the study at any time without giving a reason, even when parental consent has already been given.

I do/do not* consent for my child (Name): _____

Form: _____ to take part in this study.

* please delete as appropriate

Name of parent or guardian: _____

Signature of parent or guardian: _____

Date: _____

Phone number: _____ (day)

_____ (evening)

If you are willing for your child to participate in this study, please complete the following questions on the following pages. When you have completed the form, please return it to the school in the envelope provided as soon as possible.

PARENT OR GUARDIAN SCREENING AND CONSTENT FORM

1. Does your child have or is there a family history of any of the following conditions?
(the conditions listed here are the exclusion criteria for the study)

Please tick (√) one box or two boxes in every row

	Yes, child has condition	Yes, family history of condition	Neither
Sickle-cell anaemia			
Haemophilia			
Thalassaemia traits			
Any other blood disorder (please specify)			
Colour blindness			
Diabetes			
Any other glucose tolerance (please specify)			
Any other chronic disease (please specify)			

2. Which of the following do you think best describes your child's ethnic origin?
Please tick (√) ONE box

White Caucasian		Pakistani	
Black-Caribbean		Bangladeshi	
Black-African		Chinese	
Black-Other		Asian-Other	
Indian		Other, please specify	

3. Is English your child's first language?

Please tick (√) ONE box

Yes ☐

No ☐

If NO:
Please write down your child's first language: _____

PARENT OR GUARDIAN SCREENING AND CONSTENT FORM

KINGS College LONDON
University of London

4. How often does your child have breakfast?

Please tick (√) ONE box

Never ☐

Once a week ☐

Twice or more a week ☐

Every day ☐

Other, please state _____ ☐

If your answer is NEVER, please go directly to question number 6.

5. If your child has breakfast (even if not every day), please answer the following questions:

a. What does your child typically have for breakfast?

FOOD/BEVERAGE	AMOUNT

b. Please write down the time that your child has breakfast: _____

6. Please write down your child's birth weight.

stones: _____, pounds: _____, OR kilos: _____

PARENT OR GUARDIAN SCREENING AND CONSTENT FORM

7. Please write down the age and occupation of yourself and (if applicable) your partner, noting whether you work as an employee or are self-employed, and if your work involves supervising others.

	Father	Mother
Age (years)		
Occupation:		
Type of Industry		
Occupation title		
Occupation description		
Employee/self-employed		
Supervisory (Yes/No)		

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE

ALL INFORMATION IS CONFIDENTIAL, FOR RESEARCH PURPOSES ONLY

Please enclose this form in the envelope provided and return it to the school as soon as possible.

II.3 Participants: study information sheet, consent form

INFORMATION SHEET FOR PARTICIPANTS

BREAKFAST EATING AND LEARNING ABILITY IN ADOLESCENTS

We would like to invite you to take part in our research looking at breakfast eating and how well you learn. You should only take part if you want to. If you choose not to take part, you will not be affected in any way. Before you decide whether you want to take part, it is important for you to understand why this research is being done and what you will be asked to do. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. If you do decide to take part, please let us know beforehand if you have been involved in any other study during the last year.

Why are we carrying out the research?
Recent research shows that school-aged children who eat breakfast have better school attendance and better grades. However, the studies do not all agree on the effects of eating breakfast. Positive effects of breakfast eating on learning ability may depend on the type of breakfast rather than simply whether or not breakfast was eaten. We want to know if particular types of breakfast are related to better performance in tests of learning ability. We also want to know if mood is effected by eating breakfast and if it has an effect on the tests of learning ability.

Who will take part in the study?
All children aged 11-14 in your school will be asked to take part. Your school was selected because the children who attend come from a wide variety of backgrounds. You will be allowed to take part when you and your parents or guardians have signed consent forms saying that you understand what the study involves. You will not be allowed to take part in the study if you have anaemia or diabetes. If you start the study but then change your mind, you are free to stop at any time without having to give a reason.

This study has been approved by the King's College Research Ethics Committee, reference 04/05-51

INFORMATION SHEET FOR PARTICIPANTS

What will you be asked to do?
If you agree to take part in the study, you will be measured for height and weight and interviewed individually by either Miss Renata Micha, or Miss Julia Forbes, or Miss Kathryn Lowes about what you usually eat (for example, how often you eat breakfast, if you are a vegetarian), your physical activity, and your current health. You will be asked to complete tests of learning ability (for example, tests of memory and concentration) and questions about mood (for example, if you are tired, hungry, happy or sad). We will take two finger prick blood samples, one before and one immediately after completing the tests and the questions about mood. We will measure the amount of iron in your blood and your blood glucose levels. We want to measure these because iron and glucose levels in blood (as well as breakfast) may affect mood and the outcome of the tests. There is no risk of infection when the finger prick blood samples are taken, since all the equipment we use is sterilized.

Who will see this information?
Only the people from King's College London who are directly involved in the research will see the information about you. The information collected will not be shown to anyone else. All the information collected will be kept strictly confidential. You will not be identifiable in any verbal or written reports about the research. At the end of the study, you and your parents or guardians will be told about the results of your own tests and whether in general the type of breakfast that is eaten affects performance levels.

When will the work be carried out?
The study will be carried out between May and June 2005 by Miss Renata Micha, King's College London. If you have any questions about the study, please ring 020 7848 4594.

This study has been approved by the King's College Research Ethics Committee, reference 04/05-51

PARTICIPANT CONSENT FORM

Breakfast eating and learning ability in adolescents

I have read and understood the study information sheet overleaf.

I understand that I may freely withdraw from the study at any time without giving a reason, even when consent has already been given.

I do/do not* consent to take part in this study.

Name: _____
Form: _____

* please delete as appropriate

Have you been involved in any other research in the last year?
Yes No

Please circle

Signature of participant: _____

Date: _____

Please enclose this form in the envelope provided and return it to the school.

II.4 Administration protocol

Write down the time that the child enters the room

Hallo _____ (name of the child). My name is Renata and I am from the Department of Nutrition, of King's College, London. I am here today to see if the breakfast that you eat affects how well you do at school. The first thing I would like to ask you is whether you had breakfast today, and apart from your breakfast what was the last thing you had before you came here.

Ask the questions from the researcher's booklet, and decide whether to continue or not, according to the acceptance rule.

If NOT, explain why (will affect the results of your tests) and instruct the child to come back in 30-45 minutes, and not to have anything else in the meantime. Otherwise, we will not be able to see whether the breakfast they have affects how well they do at school, and consequently they won't be able to take part in the study.

If YES
First of all, I am going to measure your height and weight. Then, I am going to take a finger prick blood sample, in order to measure your glucose and iron in your blood. After that I am going to ask you to complete some tests for me, and some questions about your how you are feeling. I am carrying out these tests as part of the "breakfast and your brains" study. These tests are not a test of how intelligent you are, they will only measure your memory and concentration. We won't be comparing the results with your friends. We won't be showing the results to your teachers. We just want to see how you do on each test, and if this is affected by the breakfast you have. After the tests, I will take another finger prick blood sample, and then I am going to ask you a few questions about what you usually eat, your physical activity, and your current health.

1ST PART
So, let's measure your height and weight. Please take off your shoes, so that I can have a more accurate measure of your height and weight
Please take off your jumper/ jacket and empty your pockets (If they are wearing any heavy clothing, jacket or jumper).

Measure height and weight

2ND PART
Now, I would like to take the first finger prick blood sample. There is no reason for you to be afraid, since it shouldn't hurt that much. I've had a finger prick blood sample taken and it only hurts a little bit/ much less than I thought it would.

Make the child feel comfortable by asking him/her e.g. if they have any brother, or sisters, and take the first finger prick blood sample.
Well done! You are very brave. It didn't hurt you that much, did it?

3rd PART – MOOD SCALES (BEFORE)
This is the booklet that has the tests I am going to ask you to complete for me.

Give the booklet to the child

Please do not turn over the page or write on your answer booklet, until I tell you to. OK?

Firstly, I would like you to write today's date and the time on the front cover of your booklet. Today's date is _____ and the time now is _____.

Pause for the child to write

Did you write that in? Well done.

Before we do the learning tests, I would like to have an idea of how you are feeling at the moment. Please turn to page 2 of your booklet. This is a list of words that describes feelings and moods that people have, like hungry, happy and so on.

I would like you to rate each word according to how YOU feel at this moment, using the scale shown in your booklet. For example, if you <u>don't</u> feel friendly <u>at all</u>, write 0 next to the word friendly, 1 if you feel <u>slightly</u> friendly, 2 if you feel <u>moderately</u> friendly, 3 you feel if <u>very</u> friendly, and 4 if you feel <u>extremely</u> friendly.

Please read each word carefully and write in your answer. If there is a word that you do not understand or are not sure how to interpret, please ask me.

Do you understand what you have to do? Please work through the list quickly. It should only take you a few minutes. You may start.

Record start time and finish time

Well done! Thank you for that.

4TH PART – COGNITIVE FUNCTION TESTS
The next part is to do the learning tests. I want you to try to do your best. Some of the tests are easy and some are a bit harder. I do not expect you to be able to complete them all – I can't!

<u>Please do not say anything during each test, unless you are asked to do so.</u> <u>If you need to ask a question, please do so only between the tests.</u>

I will give you instructions. Again, please do not turn over the page or write on your answer booklet, until I tell you to.

<u>4.1 Word generation task</u>
The first test is a memory test. Please, turn to page 3. In this task you don't need to write anything down, since I am going to record your responses. I am going to ask you to say out loud as many words as you can beginning with a certain letter, which I will tell you in a moment. When I say GO you will have 2 minutes to say as many words as you can beginning with that letter. When the 2 minutes are up, I am going to say STOP, and you should stop. Do you understand? Please ask any questions now as you must not ask during the test.

Ready? *Start recording*

The letter is 'S'. GO!

Time 2 minutes

STOP! Well done

You can have a short rest, while we get ready for the next task.

Time 30 sec

<u>4.2 Word recall (immediate)</u>
The next test is another memory test. I am going to show you a list of 15 words for 45 seconds, which I want you to study carefully. Afterwards, I will ask you to write down as many of these words as you can remember. I am going to give you 2 minutes to write down as many as you can. Do you understand? Please ask any questions now as you must not ask during the test.

Turn over to page 4. Ready? GO!

Time 45 seconds

Stop! Turn over the next 2 pages. When I say go, write down as many words from the list as you can remember. OK?

GO! Time 2 minutes

Stop! Well done!

Rest - *Time 30 seconds*

<u>4.3 Stroop task</u>
Now, would you like to turn to page 7 of your booklet. The test we will do now is called the Stroop task. In this test, I will show you some words and you have to name the color of the ink, and NOT the word you read. To practice, I would like you to say out loud the color of the <u>ink</u> of the words you see, moving across each row from left to right. When you reach the end of one row, go to the beginning of the next one, and so on.

Let the child read the coloured words. Make sure that he/she has understood what they are supposed to be doing.

Well done! This is exactly what you have to do for the test. Do not turn over the page.

First, I am going to give you an easier test to do. The next one is going to be a bit more difficult. I want you to name as quickly and as accurately as you can the colour of the ink, and not the word you read. I would like you to do that for all the words you see in the page, moving across each line

from left to right. I am going to record your answers. Do you understand what you have to do?

When I say GO name the colour of the ink as quickly and as accurately as you can, and go onto the next word in the row. Do that for all the words in the page. Let's do the easier test first. When you finish this first test, do not turn over to the next page, until I tell you to. OK?

Start recording

Turn over to page 8 of your booklet. Ready? GO!

Time the child

(If they make many mistakes -5-6 errors in a row- instruct them again)

Well done! Now let's do the next one. Turn over to the next page. Ready? GO! (if you can, time the in-between time)

Time the child

Well done!

Rest

Time 30 seconds

4.4 Matrices

Please now turn to page 10. Look at the first page. This puzzle has been solved for you. There is a missing shape in the square at the top of the page. The test is to find which of the shapes 1, 2, 3, 4, 5 or 6 you think fits best, and then write the number of the shape in the empty square. Number two is the answer as it has a whole line (not a broken one) like the shield, but is the same shape as the circle. Can you see that?

Show that to the child

Let's look at the next one (page 11). Do you agree that shape number 3 is the answer? The larger square is the missing shape. Each row then has one square, one circle and one triangle.

Do not turn over to the next page yet. There are more puzzles to solve on the next 16 pages. I'm going to give you a few minutes to solve as many as you can. They gradually get harder, and I don't

think that anyone could solve them all. If you get really stuck on one, it is OK to leave that and go onto the next one.

Are there any questions? Turn over to the next page.
Start now!

Time 6 minutes

Stop now. Put your pencil down. Please, put a line at the bottom of this page (where they've stopped).

Well done. You can rest now, while I get ready for the next test.

Time 30-45 seconds

4.5 Speed of information processing
Now would you like to turn to page 28 in your booklet.

Make sure that the child is on right page

This is a number search test. This page explains what you have to do in this test. Do not turn over to the next page. There will be 2 pages that have several numbers. You have to circle blocks of three consecutive odd numbers.

There are two rules that I want you to remember.

(1) Each number is only allowed in one line of circled numbers.
Show the example to the child

(2) Circled numbers cannot span two lines
Show the example to the child

Now you try doing the example.

Show that to the child.

Do you understand what you have to do?

When I say GO circle as many three consecutive odd numbers as quickly as you can. I am going to give you 3 minutes to do as many as you can, on both pages. When I say stop, please put a cross through the number you are on. Turn over to page 29.

Ready? GO!

Time 3 minutes

STOP! Please put your pencil down.

Make sure that they've put a cross where they've got up to on the page

Well done! Let's have a rest now.

4.6 Serial Sevens

Now please turn to page 31 of your booklet. We are now going to do another memory game. I will give you a number and I want you to subtract 7 from that number and say the answer, and then subtract seven from that number and say the next answer, and so on. For example, 663 - 656 - 649 - 642 - - Now you carry on 635 - 628 - 621 - 614 - 607 - 600 - 593 - 586, etc (perhaps describe the tests "663 minus 7 is 656, minus 7 is ....."). You are not allowed to use your fingers to count down, and please don't count aloud.

I will give you the number, and when I say go you can start subtracting by 7 out loud, as quickly and as accurately as you can. I am going to give you 3 minutes to make as many subtractions as you can. I am going to record your answers, so you don't need to write anything down. Do you understand what you have to do?

Start recording, and get ready to write down the answers.

Are you ready? The number is 857. GO!

Time 3 minutes.

Stop! Well done! I know that wasn't easy.

Rest

Time 30 seconds

4.8 Word recall - delayed

The next test is another memory test. Do you remember the list of the 15 words that I showed you about 20 minutes ago? I showed the list to you for a few seconds and then you had to write down as many words as you could remember.

Make sure that the child has understood which test you are talking about.

I would like you to try to remember these words. I am going to give you 2 minutes to write down as many as you can. Do you understand?

Please turn to page 32 of your booklet. When I say GO, write down as many words from the list as you can remember.

Ready? GO!

Time 2 minutes

Stop! Please put your pencil down.

Well done! We are now finished with learning tests.

Rest - *Time 30 seconds*

5th PART - MOOD SCALES (AFTER)

Now, let's do the mood scales again. I would like to get an idea of how you feel now, after having completed the tests. Please turn over to page 33 of your booklet. You can see the same lists of words that you saw before. I want you to rate these words again, according to how you feel right now/at this moment. Do you understand what you have to do? Again, this should only take you a few minutes. Please, work through the list quickly. You may start.

Record start time and finish time

Have you finished? OK then. Let's move on to the next part

6th PART - TASK DEMAND

Now, I would like you to tell me how difficult you thought the tests were. Please, turn over to page 34 of your booklet.

There are three questions for each one of the tests you did. I want you to rate the tests, for each one of the questions. The rating scale is similar to the one we used for the words that describe people's feelings.
Let's look at the first question.

Say the first question and explain the rating scale for it

I am going to remind you briefly about each of the learning tests.

Remind the child about the learning tests/ Make sure they remember the individual tests.

I would like you now to rate the test for the first question. Please, work through the list of tests quickly. Do you understand what you have to do? If you are not sure about any of the tests ask me, and I will remind it to you again.

OK then. Let's do the first question. Please, work through the tests quickly. Let ,e know when you've finished.

Record start time

Have you finished? Let's look at the next question. Please, turn over the page.

Say the second question and explain the rating scale for it

Now, I want you to rate the tests according to how much effort you put into them. Again, if you are uncertain about any of the tests, please ask me.

OK? Let's do this question then. This should not take you too long.

Have you finished? Let's look at the third question. Please, turn over the page.

Say the third question and explain the rating scale for it

Now, I want you to rate the tests according to how tiring you thought they were.

OK? Let's do this question then. This should not take you too long. Let me know when you've finished.

Record finish time

Well done!

7th PART - FINGER PRICK
Now, I would like to take the second finger prick blood sample. This will be just like the first finger prick.

Make the child feel comfortable and take the second finger prick blood sample.

Well done! You are indeed very brave!

Now you can rest for a little while, until we get ready for the last part.

Rest - Time 1 minute

8TH PART - INTERVIEW QUESTIONS
Now, I would like to ask you a couple of questions about what you usually eat, your physical activity, and your current health. This shouldn't take us too long. So, let's start ok?

Interview the child

Thank you very much for that. You did very well! Before you go I would like to ask you a small favour. Please, do not discuss what we did here today and the tests with your fellow pupils, until the entire testing/ study has been completed. If you do that, then we will not be able to see if the breakfast you eat effects how well you do at school. Can you do that for me? Thank you very much.

This is a certificate for you to keep, that certifies that you have taken part in our study, and that you have helped us. I would like to thank you once more for taking part, and it was really nice meeting you. Once, again, you did very well!

II.5 Appointment form

KING'S College LONDON

Name: _____

Date of appointment: _____

Meet at reception at 8.30am.

Please, have your usual breakfast on the day of your appointment. After breakfast, don't eat or drink anything else (you can have water). If you don't have your breakfast you won't be able to take part.

For you to complete:

On the day of your appointment (as shown above) please fill in the table below and bring this form with you to your appointment.

Time started to eat breakfast	AM
Time finished eating breakfast	AM

Enjoy your breakfast and we look forward to meeting you.

Kind regards,

Renata, Julia and Kathryn.

II.6 Researcher's booklet

Sacred Heart R.C. Secondary School
Camberwell New Road
London SE5 0RP

Today's date: _____ (Day/ Month/ Year)

Time on arrival: _____ AM

Form class: _____

Date of birth: _____ (Day/ Month/ Year)

Gender: Male/ Female (please circle)

- Did you have your usual breakfast? Yes/ No
- At the time you usually have it? Yes/ No (if NO write the time_____)
- What was the last thing you had before you came here?

_____
_____
_____

(Probe: It doesn't have to be a meal, it could be sth that a friend gave to them, on their way to school, at school, a sweet, a candy, drink – half a can of coke, bread, milk, juice, fruit etc

- What time did you have it? _____

(Acceptance Rule: <10g CHO, time >90 mins)
If not, we postpone the appointment accordingly (after 90 minutes) and instruct the child not to have anything else, otherwise he/she will not be able to take part in the study

HEIGHT AND WEIGHT MEASUREMENTS

HEIGHT: 1)_____, 2)_____ 3)_____CM

WEIGHT: 1)_____, 2)_____ 3)_____KG

1ST FINGER PRICK BLOOD SAMPLE

RECORD TIME: _____AM

BLOOD GLUCOSE: _____mg/dL

HAEMOGLOBIN: _____g/L or g/dL

MOOD SCALES AND COGNITIVE FUNCTION TESTS

	FINISH TIME	FINISH TIME	TIME TO COMPLETE
Mood scales-before			
Word generation task			
Word recall -immediate			
Stroop task			
• Control test			
• Actual test			
Matrices			
Speed of information processing			
Serial Sevens			
Word recall - delayed			
Mood scales - after			
Task demand			

2ND FINGER PRICK BLOOD SAMPLE

RECORD TIME: _____AM

BLOOD GLUCOSE: _____mg/dL

HAEMOGLOBIN: _____g/L or g/dL

INTERVIEW QUESTIONS

TIME NOW: _____ AM/ PM

Please, remember that everything you say to me is strictly confidential, and this information will not be shown to anyone else!

1. How often do you have breakfast?

Please tick (√) ONE box

Every day ☐

Twice or more a week ☐

Once a week ☐

Never ☐

Other, please state _____ ☐

2. a. Have you had anything to drink and/or eat since you woke up this morning?
Please tick (√) ONE box

Yes ☐

No ☐

If YES:
b. Please tell me what you had and roughly what time.
(Probe: added sugar, milk semi-skimmed etc)

Description of food or drink	Time	Amount in household measures or photo

What time was it when you finished eating?: _____

3. Can you think of anything else that you had to drink and/or eat this morning?
Please tick (√) ONE box

Yes ☐

No ☐

If YES:
Please tell me what you had and roughly what time.

Description of food or drink	Time	Amount in household measures or photo

What time was it when you finished eating?: _____

4. Can you think of anything else that you had to drink/eat on your way to school?
(Probe: This might include anything that you bought or that a friend gave you)
Please tick (√) ONE box

Yes ☐

No ☐

If YES:
Please tell me what you had and roughly what time.

Description of food or drink (on their way to school)	Time	Amount in household measures or photo

What time was it when you finished eating?: _____

5. Can you think of anything else that you had to drink/eat since you came to school?
(Probe: This might include anything that you bought or that a friend gave you)

Please tick (√) ONE box

Yes ☐

No ☐

If YES:
Please tell me what you had and roughly what time.

Description of food or drink (at school)	Time	Amount in household measures or photo

What time was it when you finished eating? _____

6. **Did you have tea, coffee, coke, red bull or an alcoholic drink this morning?**
Please tick (√) ONE box

Yes ☐

No ☐

If YES:
Please tell me how much and what time:
(probe: added sugar, milk etc/ at home – on their way to school – at school)

Description of drink	Time	Amount in household measures or photo

7. **What was the last thing you had to drink/eat last night?**

Description of drink	Time	Amount in household measures or photo

What time was it when you finished eating? _____

8. a. **Are you a vegetarian?**
Please tick (√) ONE box

Yes ☐

No ☐

If YES:
b. **Do you sometimes eat fish?**
Please tick (√) ONE box

Yes ☐

No ☐

c. **How long have you been a vegetarian?**
Please tick (√) ONE box

Less than a year ☐

1 – 5 years ☐

More than 5 years ☐

Always ☐

d. **Why are you a vegetarian? (Number the boxes in order of importance from 1 to 5)**

My family is vegetarian ☐

I do not like meat ☐

I do not agree with killing animals ☐

Meat is fattening ☐

Health reasons ☐

Other, please state _____ ☐

9. **Do you follow a special diet for religious reasons?**
Please tick (√) ONE box

Yes ☐

No ☐

If YES, please give details:

_____

_____

_____

_____

10. **Are you taking vitamin or mineral tablets/ supplements now?**
Please tick (√) ONE box

Yes ☐

No ☐

If YES. Please tell me what type of supplements you take and how often you take them:

Type	How often

11. **Are you on a special diet for medical reasons?**
Please tick (√) ONE box

Yes ☐

No ☐

If YES, please describe this diet and say why you are on it:

_____

_____

_____

_____

12. **Do you take any medication prescribed by a doctor on a regular basis?**
Please tick (√) ONE box

Yes ☐

No ☐

If YES:
Please tell me what type and why:

What type	Why

13. **In the past four weeks, have you had any infections?**
Please tick (√) ONE box

Yes ☐

No ☐

If YES, please give details:

Type of infection	Why	How long did it last?

14. **On average, how often do you have the following foods or drinks?**
Please tick (√) ONE box in every row

	Never	Once a week	Twice or more a week	Once a month
Red Meat				
Other types of meat or meat products				
Chicken & turkey				
Eggs (e.g. in omelettes or sandwiches)				
Burgers				
Spaghetti Bolognese & lasagne				
Beans & lentils				
Fish or fish products (e.g. fish fingers)				
Tea				
Coffee				
Coke, Pepsi, or other cola drinks				
Red Bull				
Alcohol (beer, cider, wine, alcopops, etc.)				

15. **Have you gone on a diet to LOSE weight in the past year?**
Please tick (√) ONE box

Yes ☐
No ☐

16. **Have you deliberately tried to GAIN weight in the past year?**
Please tick (√) ONE box

Yes ☐
No ☐

17. **Has your weight changed in the past year by more than a stone (14 pounds or six kilos)?**
Please tick (√) ONE box

Yes ☐
No ☐

If YES:
Did you lose or gain weight?
Please tick (√) ONE box

Lose ☐
Gain ☐

18. **How would you describe your body weight?**
Please tick (√) ONE box

Very underweight ☐
Slightly underweight ☐
About right ☐
Slightly overweight ☐
Very overweight ☐

19. **Did you have any physical exercise today?**
(probe if you walked to school etc, anything you've done at school as well)

Please tick (√) ONE box

Yes ☐
No ☐

If YES, please tell me:
a. what time was that? _____
b. what type of exercise? _____
_____
_____
c. for how long? _____

20. **Did you have any physical exercise yesterday?**
Please tick (√) ONE box

Yes ☐
No ☐

If YES, please tell me:
a. what time was that? _____
b. what type of exercise? _____
_____
_____
_____
c. for how long? _____

21. **What time did you go to sleep last night, and what time did you wake up this morning?**
a. time you slept _____
b. time you woke up _____

22. **Do you have TV set in your bedroom?**
Please tick (√) ONE box

Yes ☐
No ☐

FOR GIRLS ONLY

23a. **Have you started your periods?**
Please tick (√) ONE box

Yes ☐

No ☐

b. **If YES, how old were you when they started?**

_____ years _____ months

c. **How often do you have your periods?**
Please tick (√) ONE box

Regularly, about once a month ☐

Irregularly ☐

24. **a. Are you having your period currently?**
Please tick (√) ONE box

Yes ☐

No ☐

b. If YES, when did it start? _____

c. If NO, are you expecting your period within the next 5 days?
Please tick (√) ONE box

Yes ☐

No ☐

25. **When was the start of your most recent period?**
Please tick (√) ONE box

This week ☐

About 1 week ago ☐

About 2 weeks ago ☐

About 3 weeks ago ☐

About 4 weeks ago ☐

26. **Are you taking contraceptive pills?**
Please tick (√) ONE box

Yes ☐

No ☐

TIME NOW: _____ **AM/ PM**

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE

ALL INFORMATION IS CONFIDENTIAL, FOR RESEARCH PURPOSES ONLY

II.7 Mood Scales, Cognitive Function tests, and Task Demand questions

Mood scales

Below is a list of words, which describe the feelings and moods that people have. Please rate each word according to how you feel at this moment, using the following scale:

0	**=**	**not at all**
1	**=**	**slightly**
2	**=**	**moderately**
3	**=**	**very**
4	**=**	**extremely**

Friendly	☐	Alert	☐
Nervous	☐	Confident	☐
Drowsy	☐	Tired	☐
Happy	☐	Angry	☐
Calm	☐	Contented	☐
Uncertain	☐	Lively	☐
Sad	☐	Tense	☐
Energetic	☐	Sluggish	☐
Muddled	☐	Clearheaded	☐
Relaxed	☐	Hungry	☐
Dissatisfied	☐	Thirsty	☐

Do NOT turn over to the next page yet

Cognitive Function Tests

Thinking of words (Word generation task)

Say out loud as many words as you can beginning with a certain letter, which I will tell you in a minute.

You don't need to write anything down.

Listen to the instructions carefully.

Do NOT turn over to the next page yet

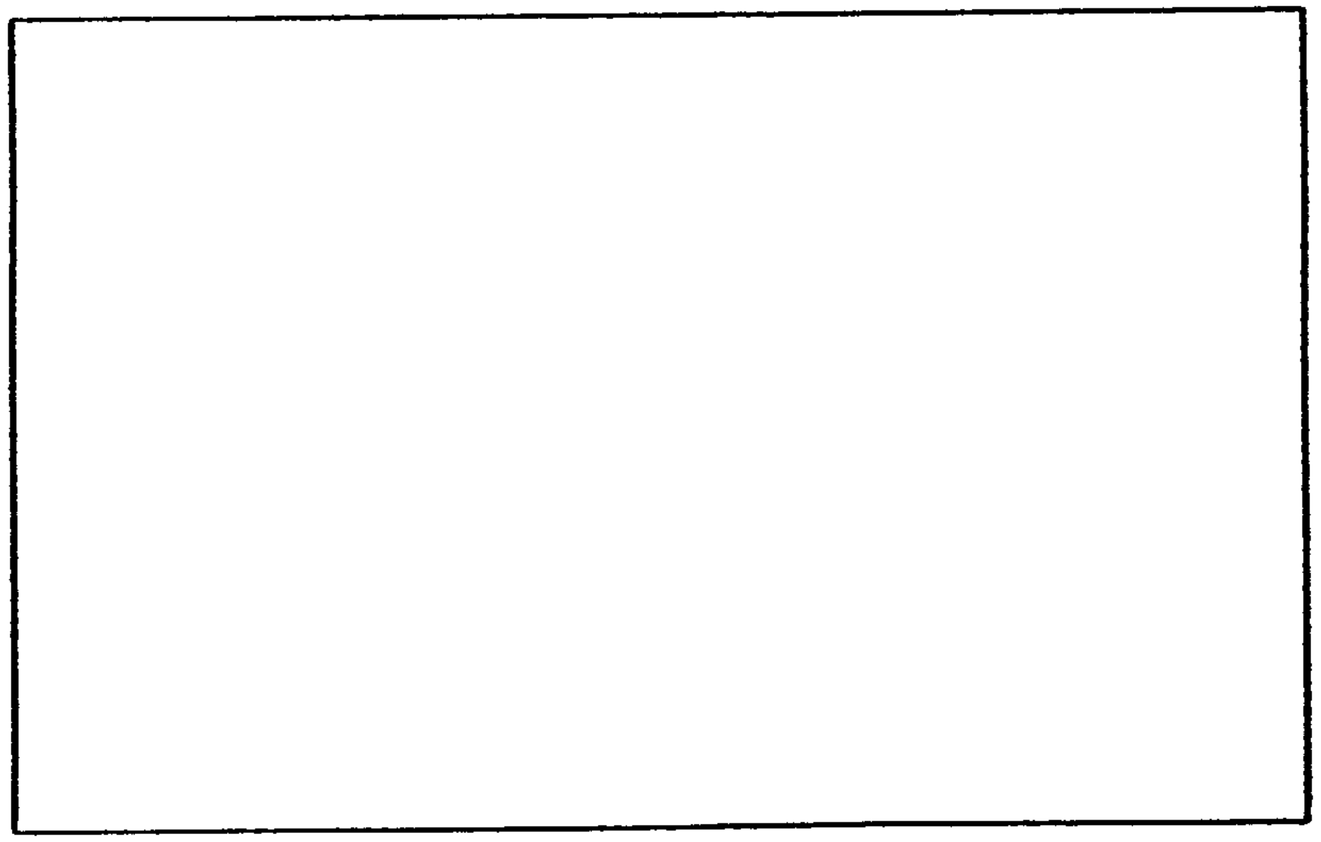
Word list

Study carefully the list of 15 words. Don't write anything down.

Product
Deed
Hide
Glory
Costume
Banner
Gallery
Breeze
Tool
Victory
Bloom
Angle
Origin
Tribute
Vision

Do NOT turn over to the next page yet

In the box below, write down as many words as you can remember from the list.



Do NOT turn over to the next page yet

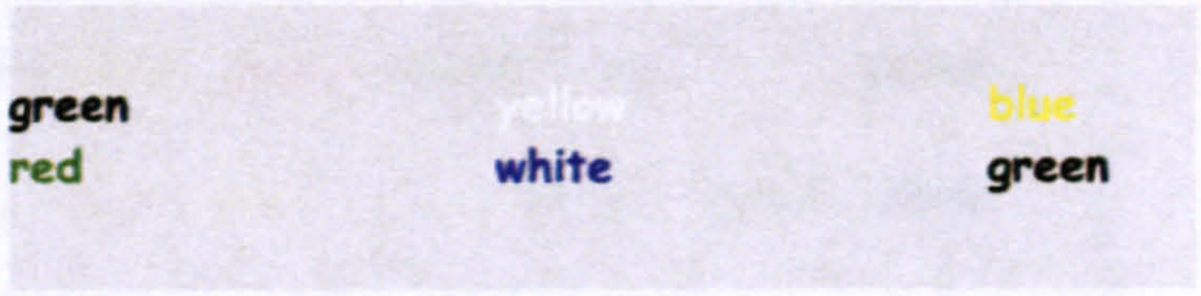
Stroop Task

Name the COLOUR of the INK, moving across each row from left to right.
DON'T say the word you read.

For example:

Easier test

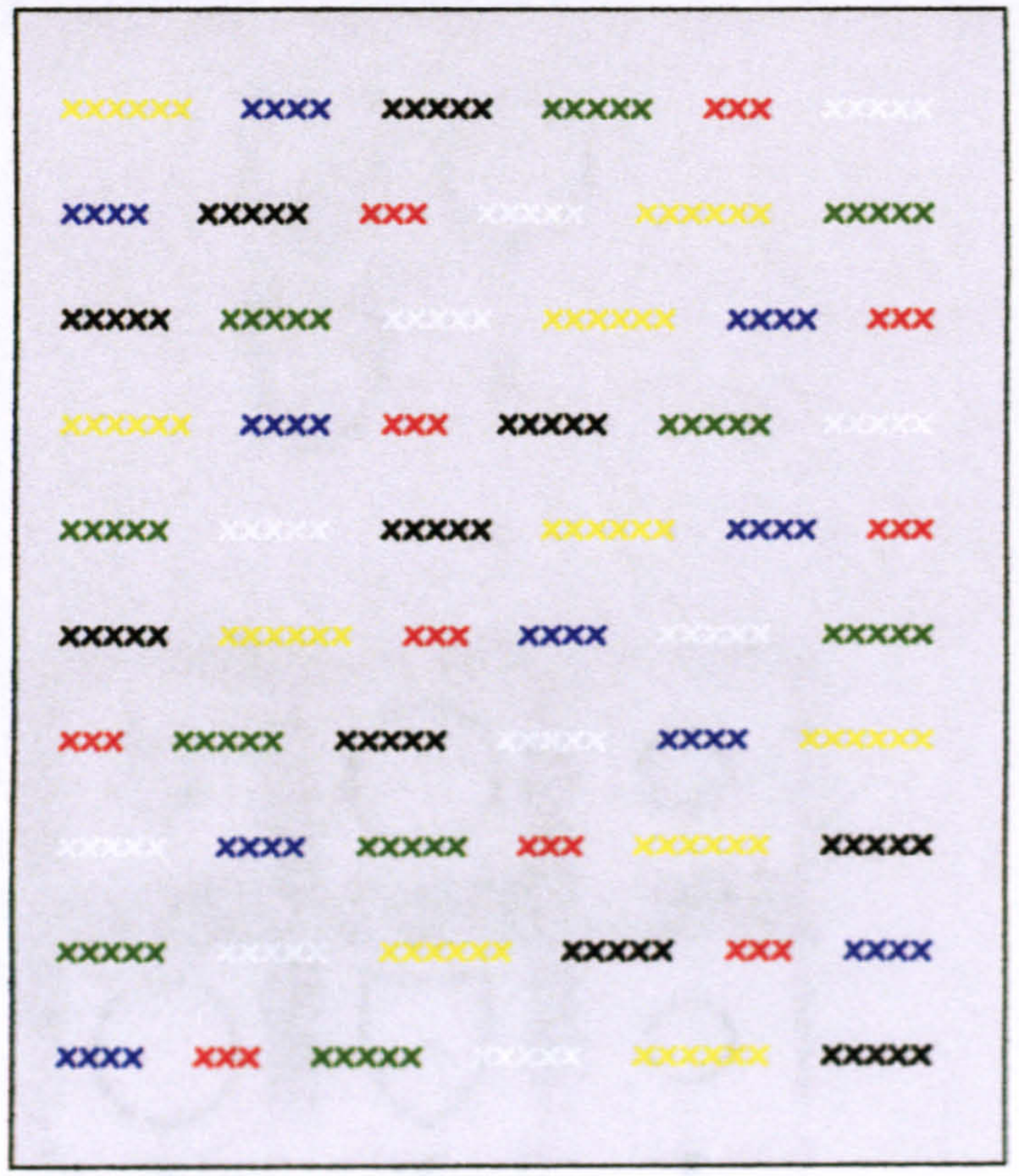
Harder test



Do NOT turn over to the next page yet

Easier test

Name the **COLOUR** of the **INK**.
Do that as quickly and as accurately as you can.



Do NOT turn over to the next page yet

Harder test

Name the colour of the **INK**. **DON'T** say the word.
Do that as quickly and as accurately as you can.

green yellow red blue white black

yellow blue black green red white

red green black white blue yellow

blue red black green yellow white

white red blue yellow black green

black green white yellow blue red

red white yellow blue green black

white green black red blue yellow

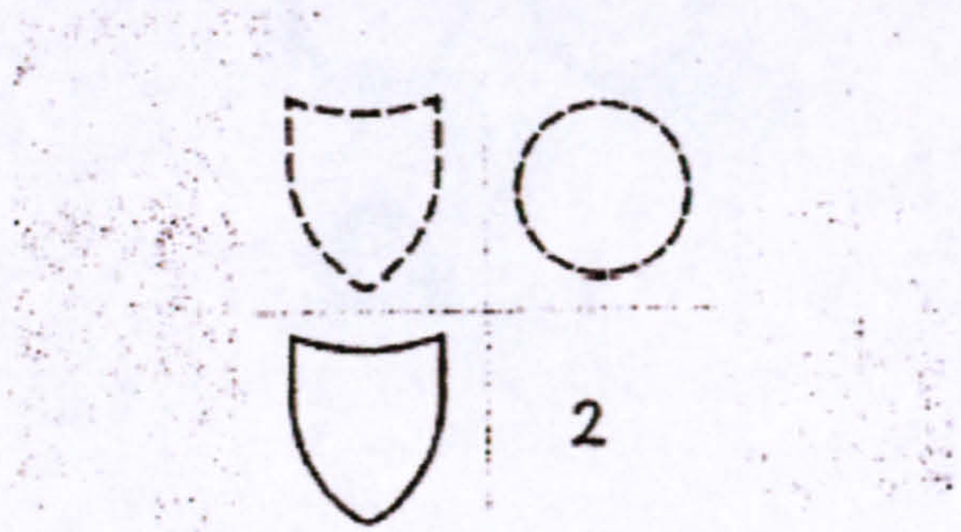
blue red green white yellow black

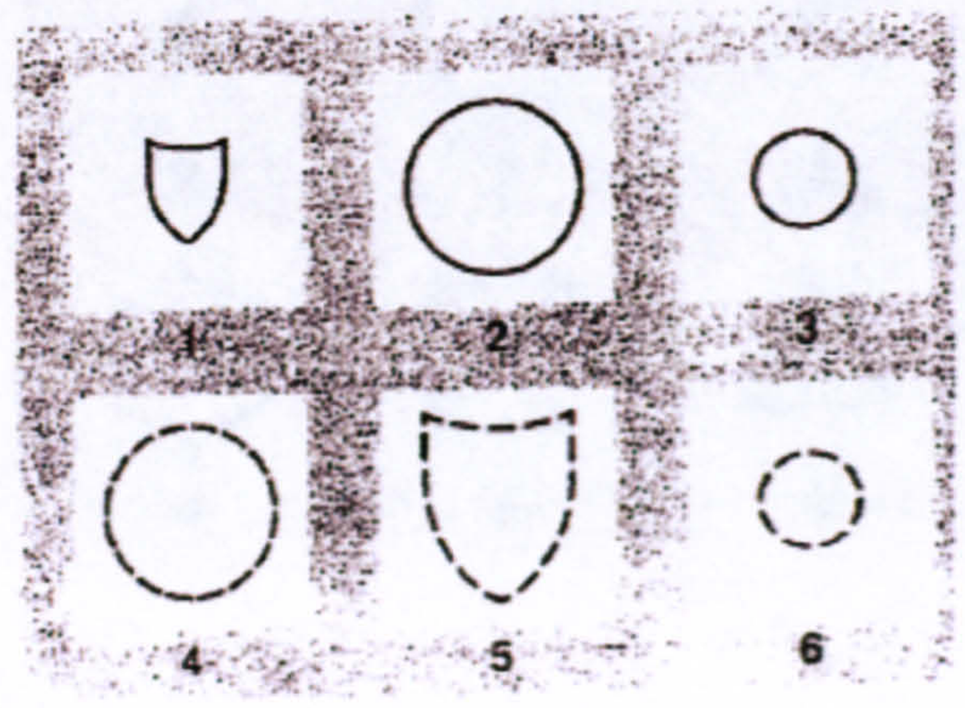
yellow white black green blue red

Do NOT turn over to the next page yet

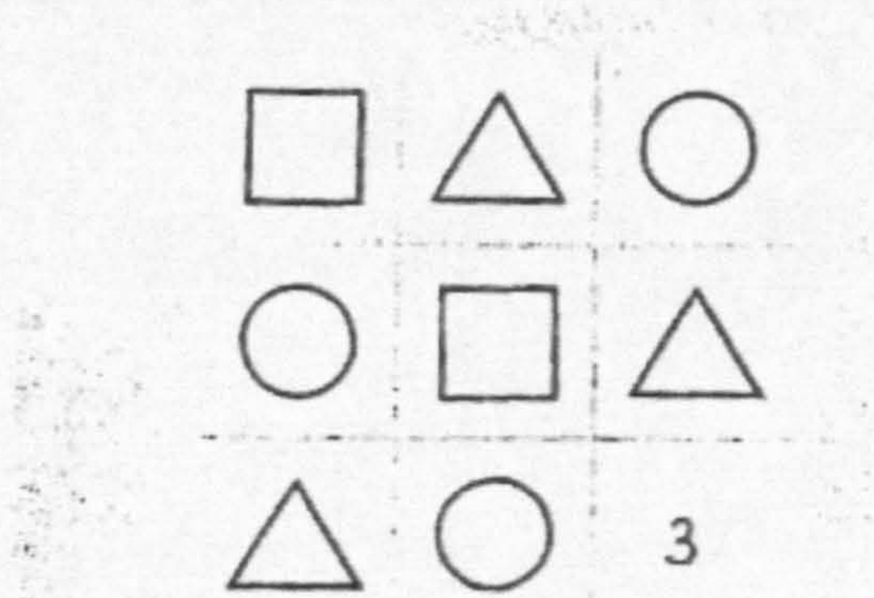
Find the missing shape

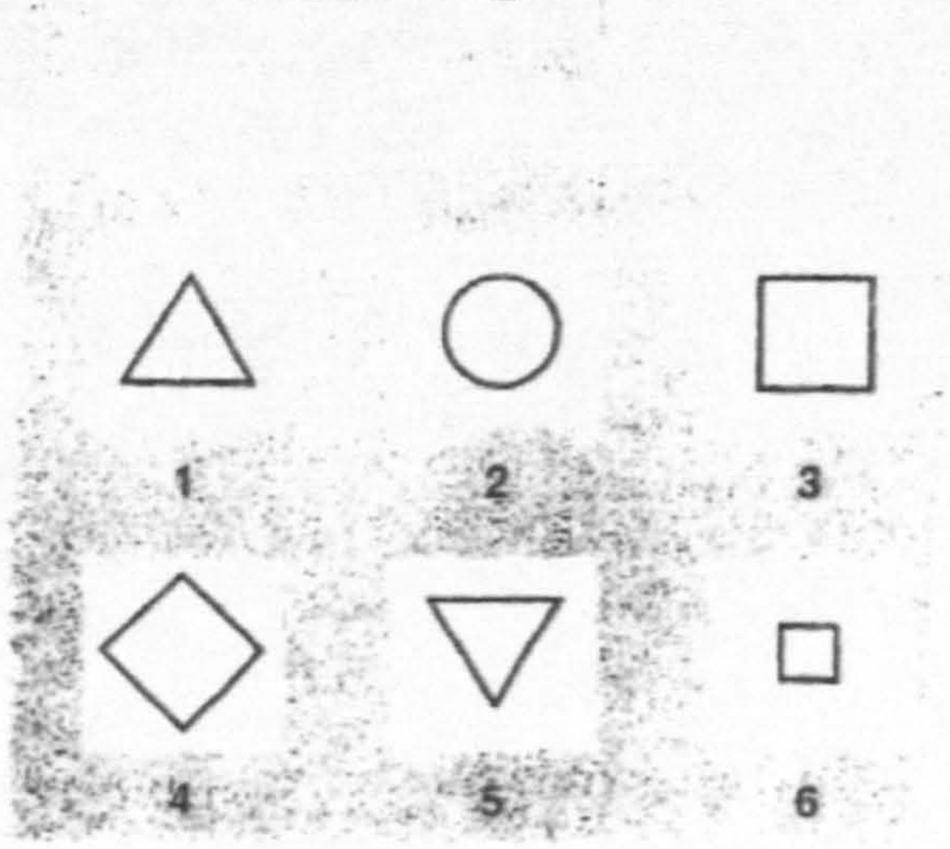
Write the number of the missing shape in the empty box, like this





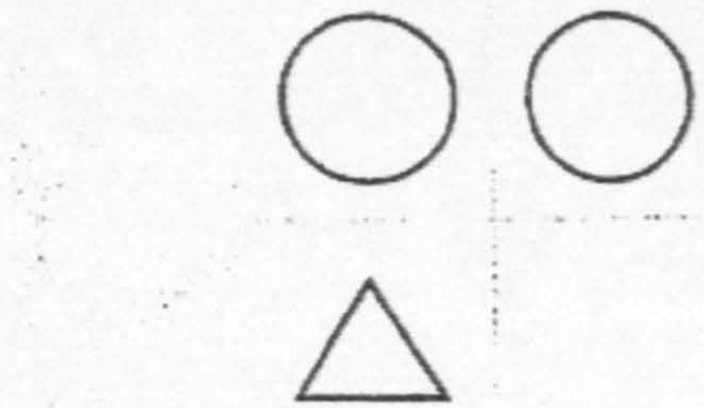
and like this

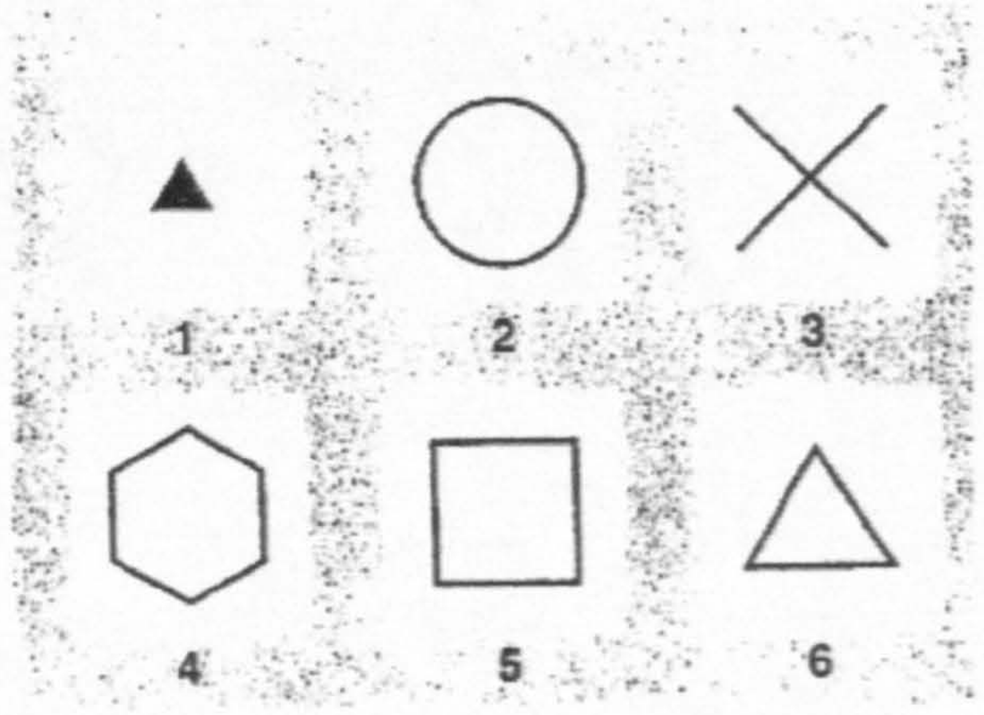


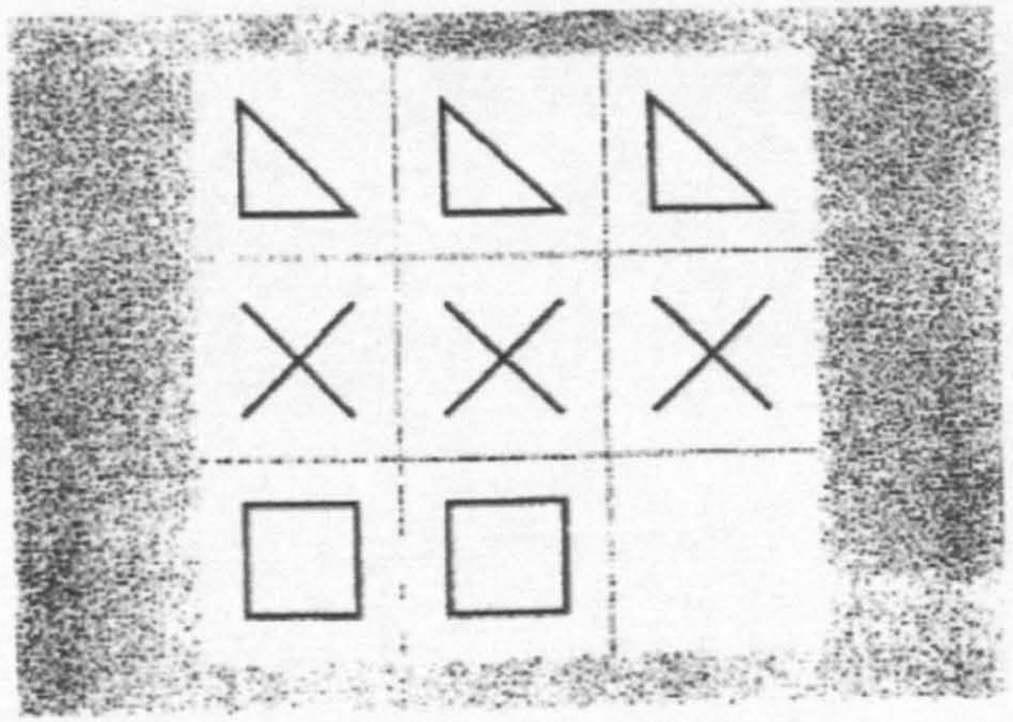


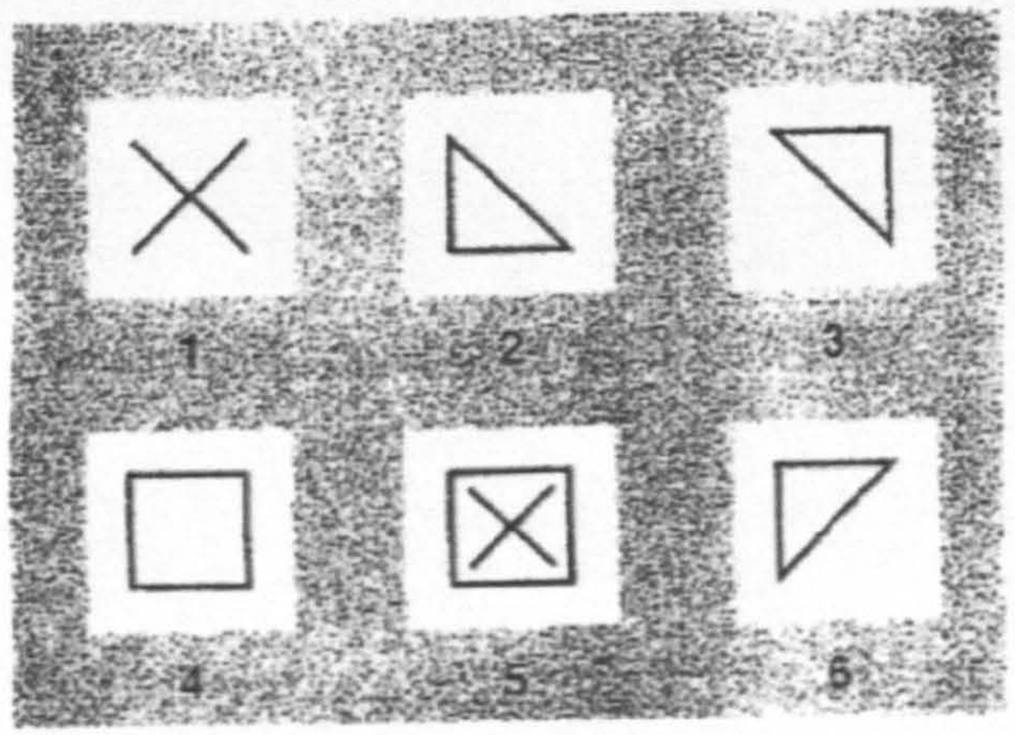
On the next 16 pages there are more of these puzzles.
They gradually get harder
Do as many as you can, as quickly as you can.

Do NOT turn over to the next page yet








1
2
3
4
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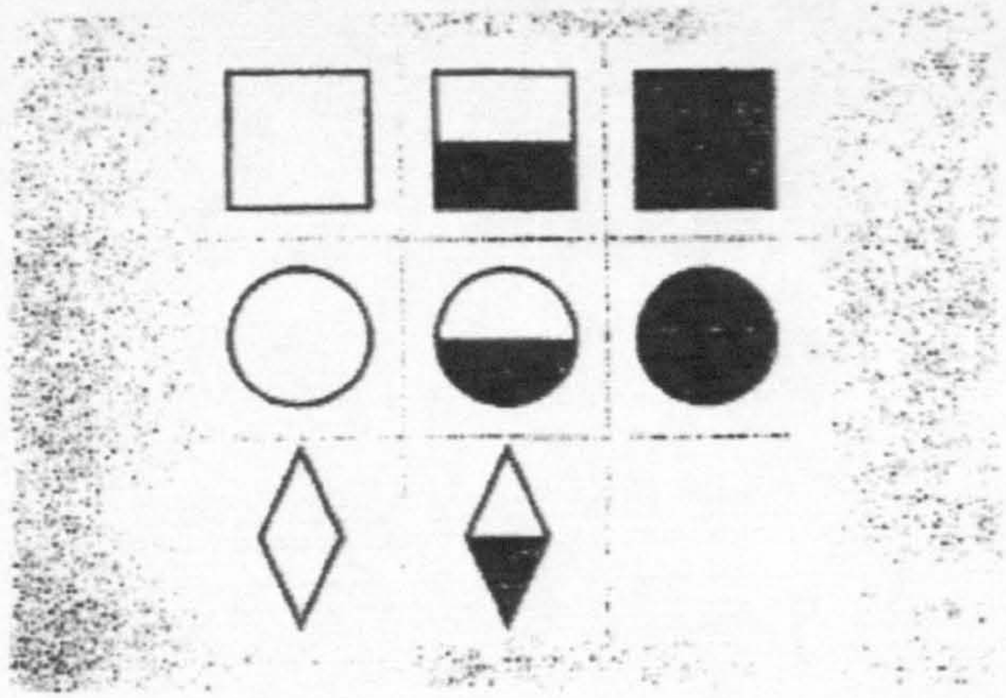

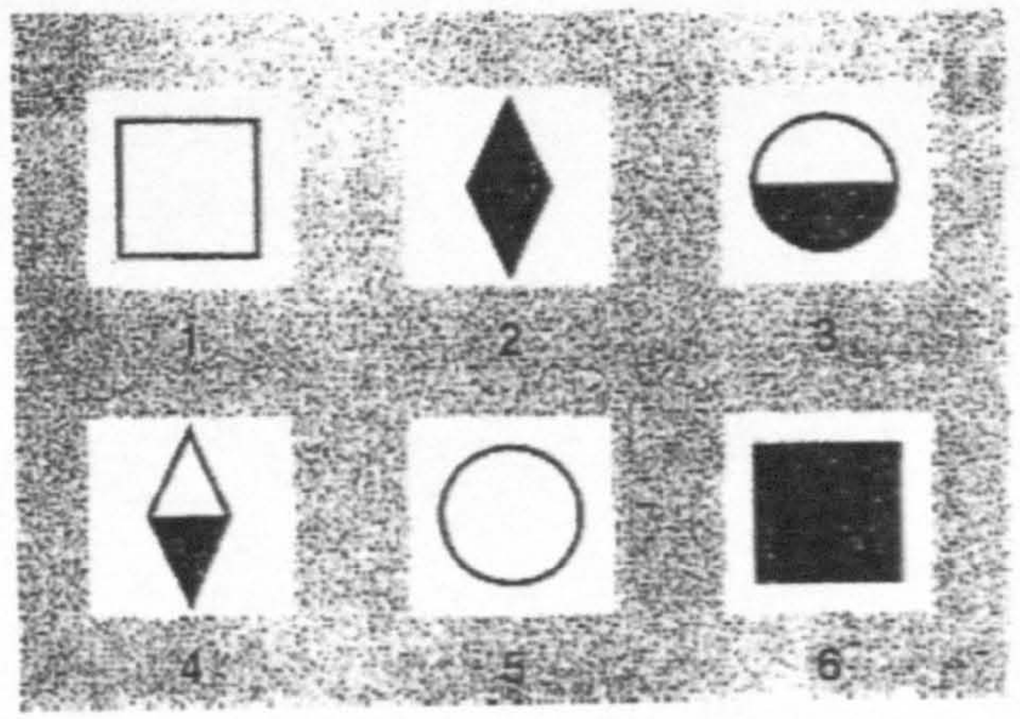
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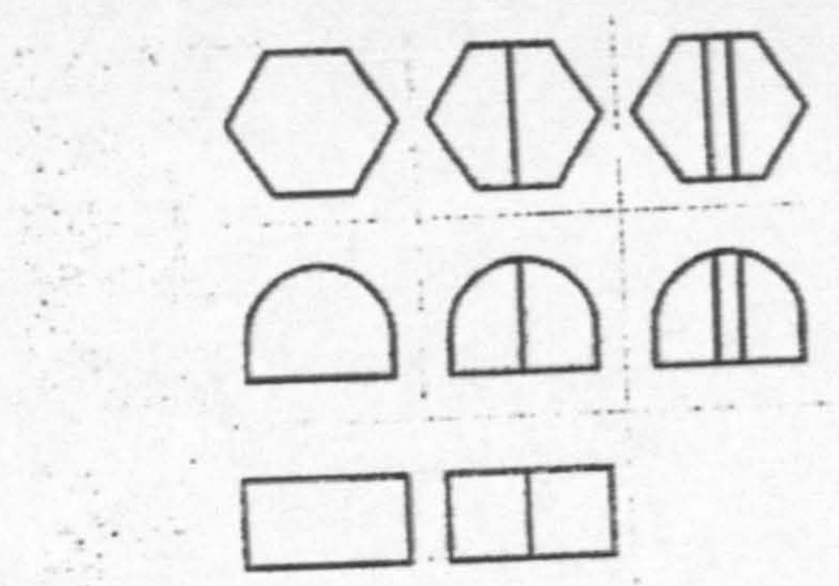

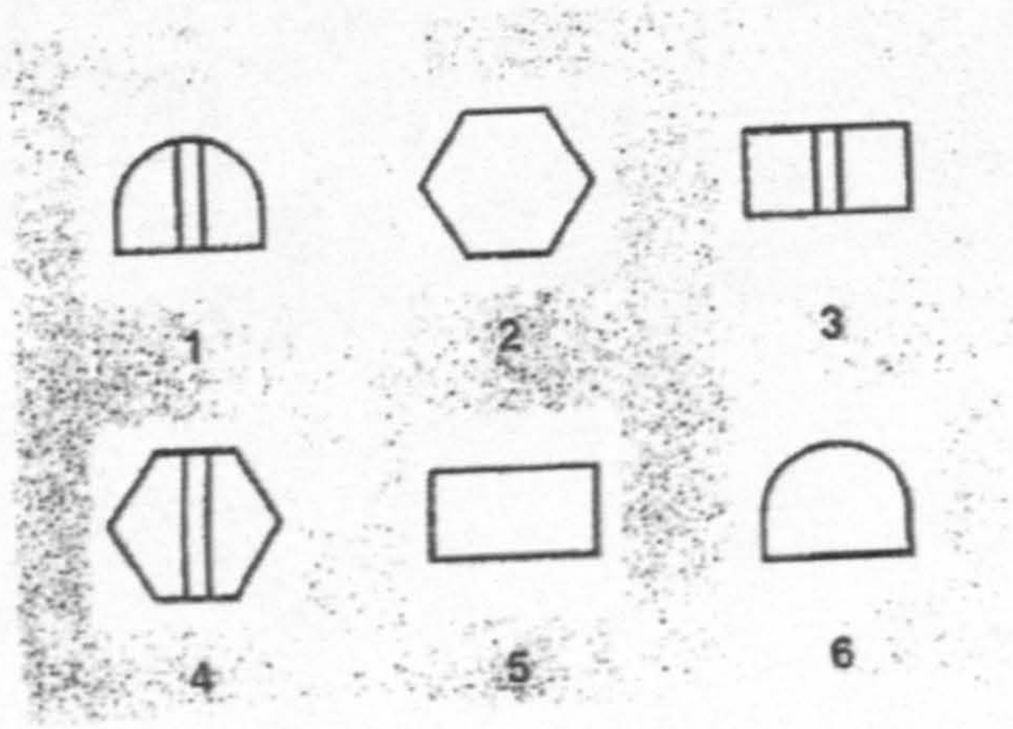
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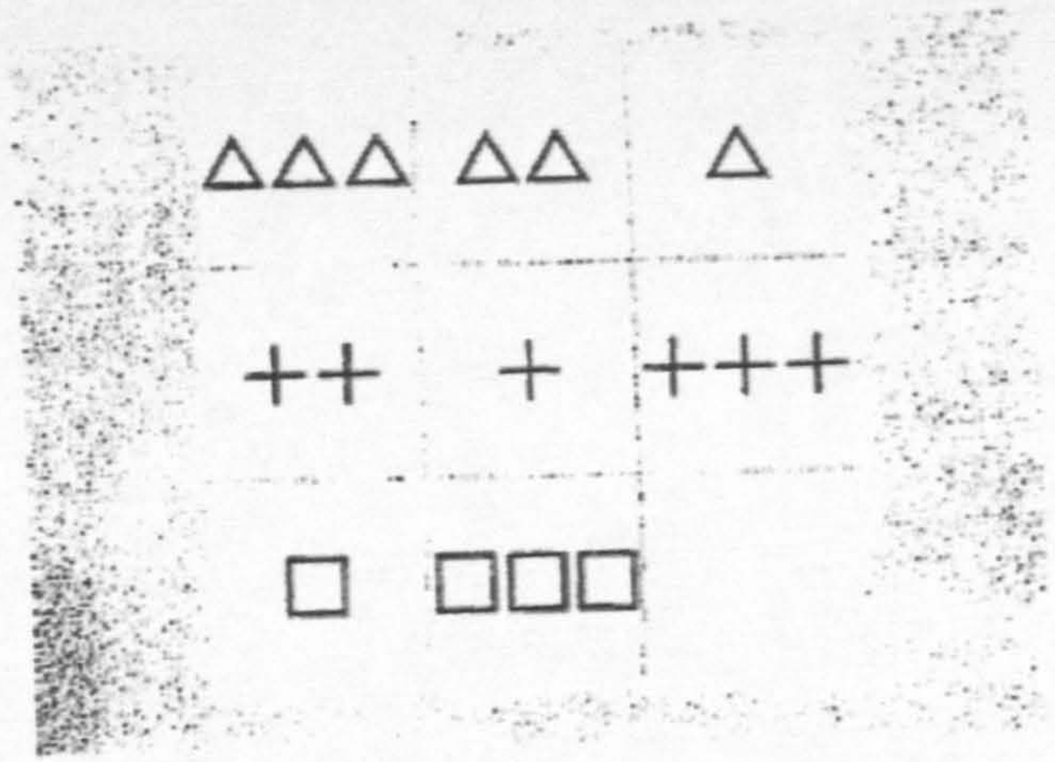

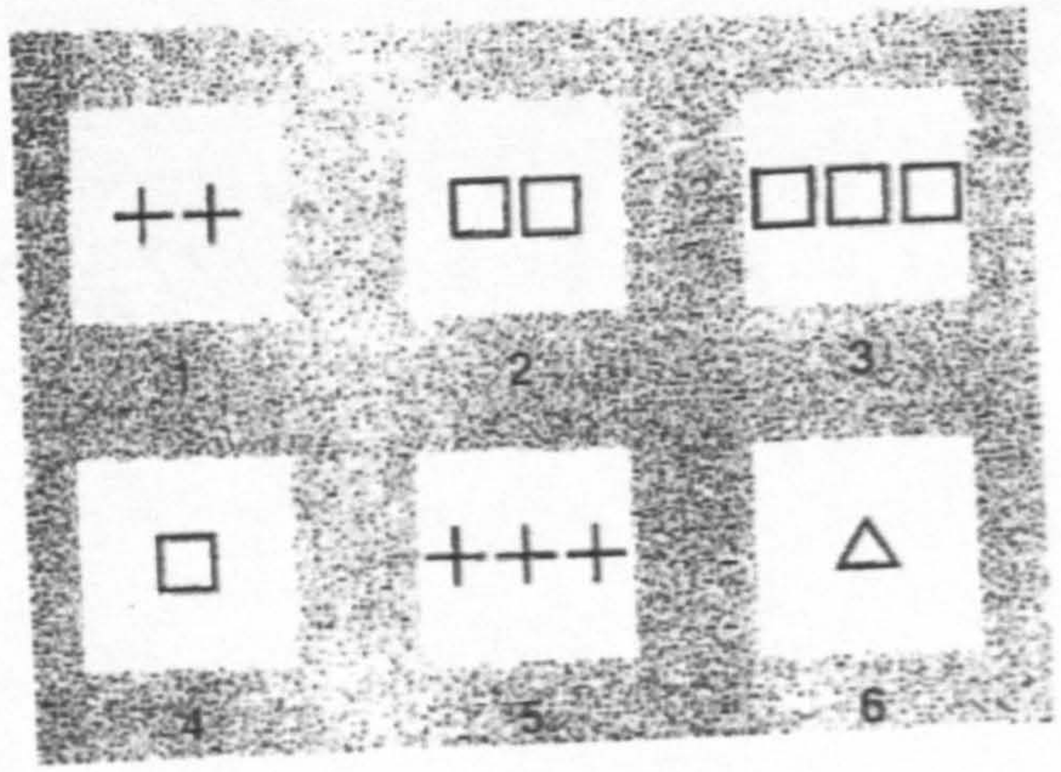
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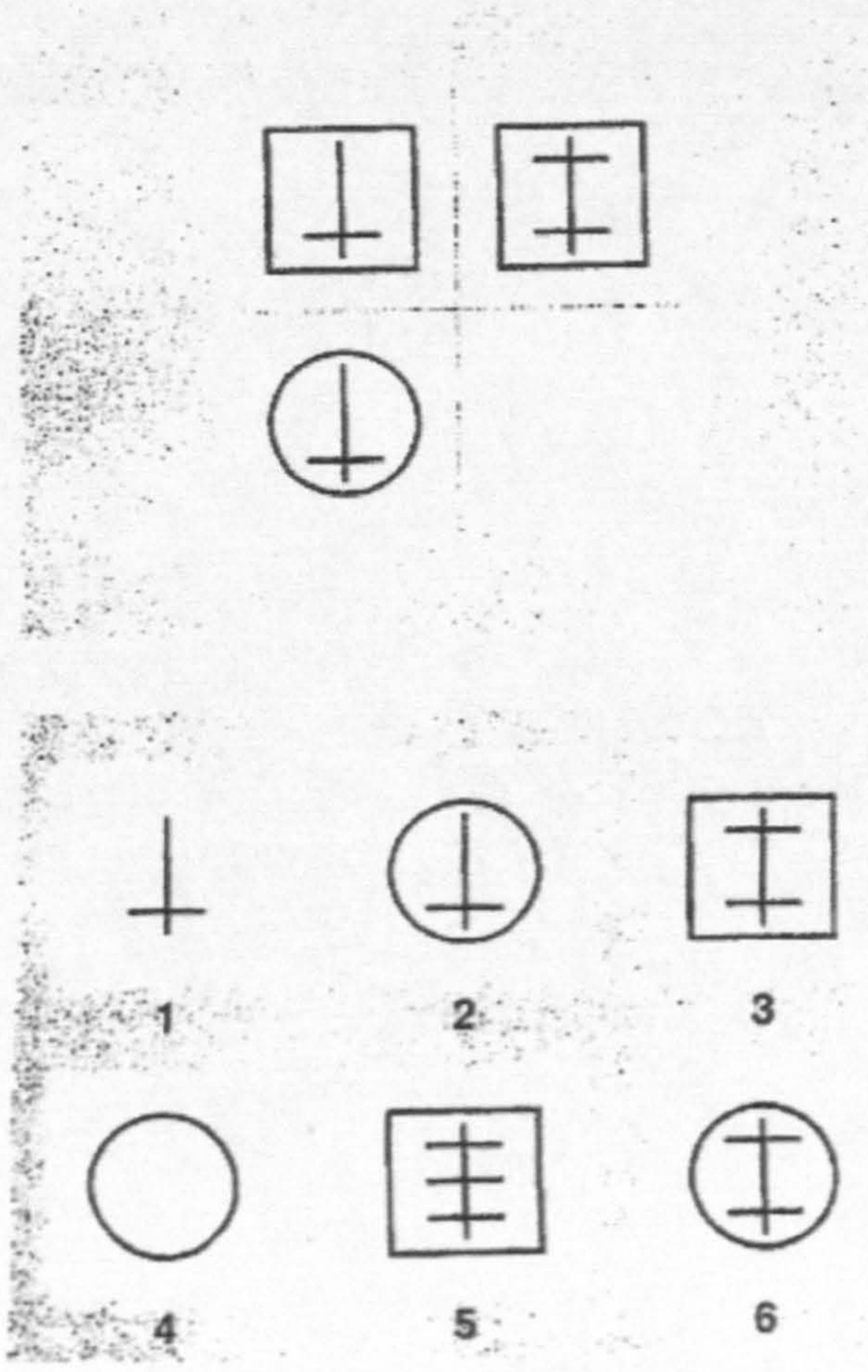
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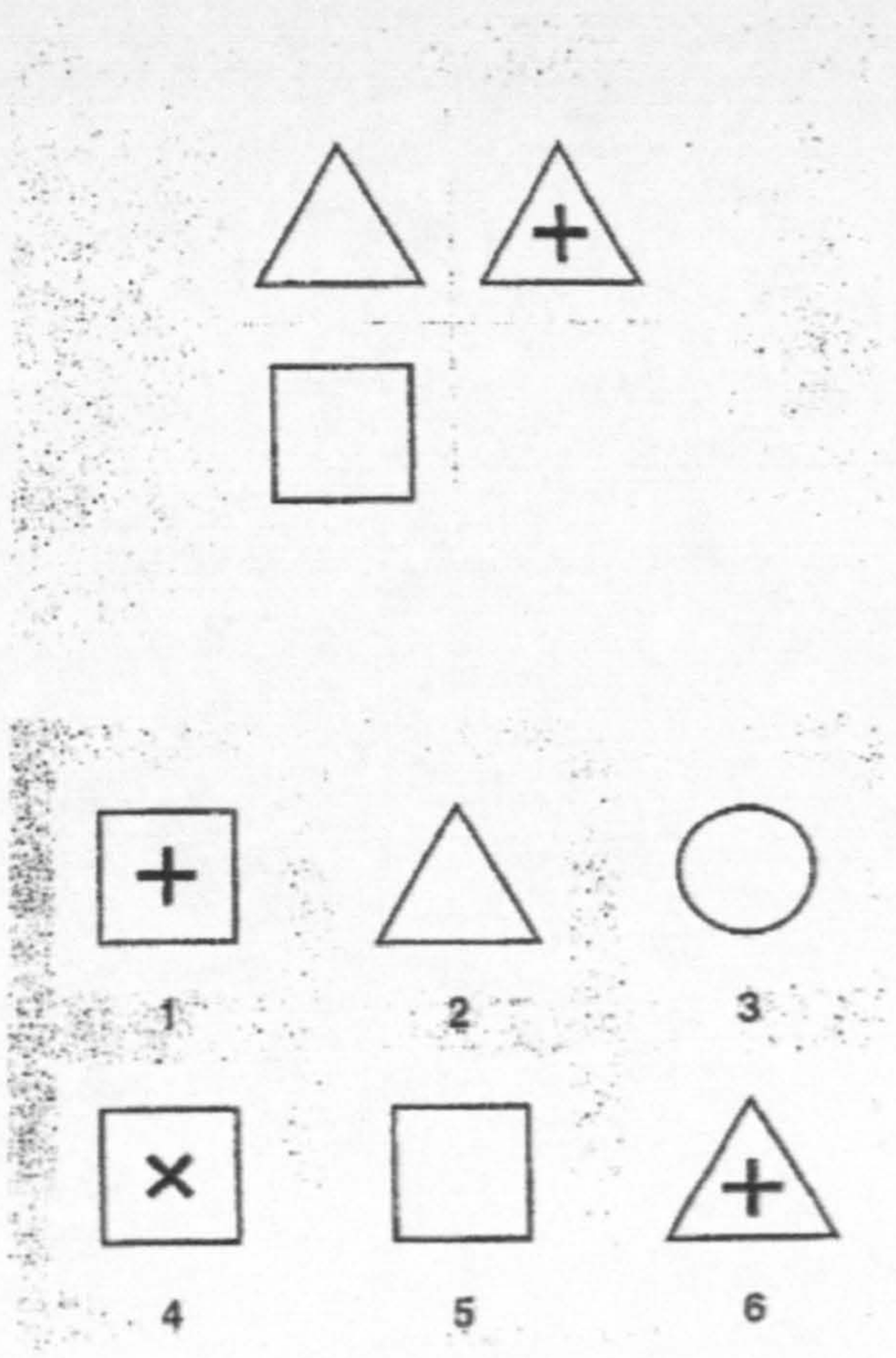
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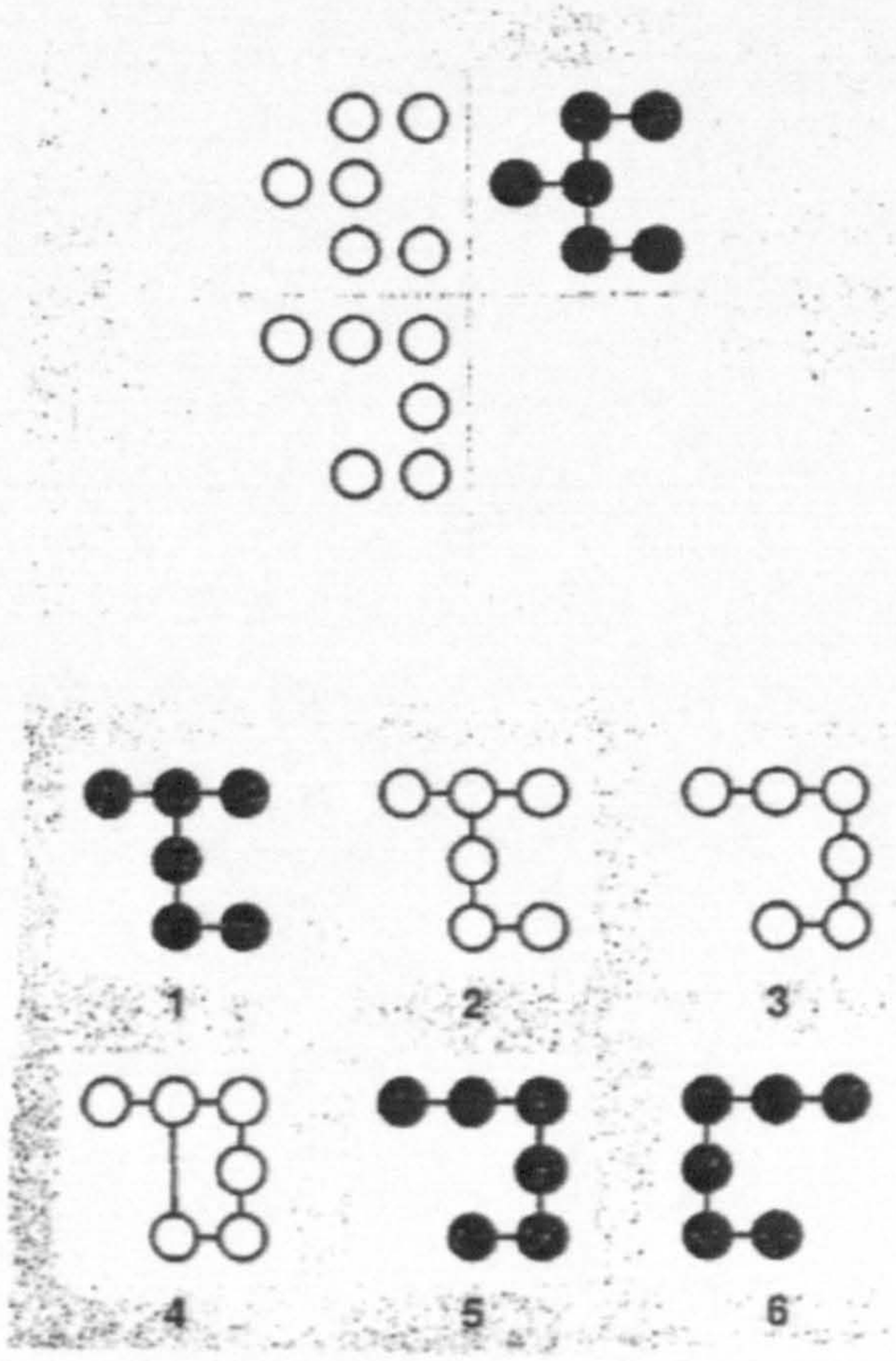
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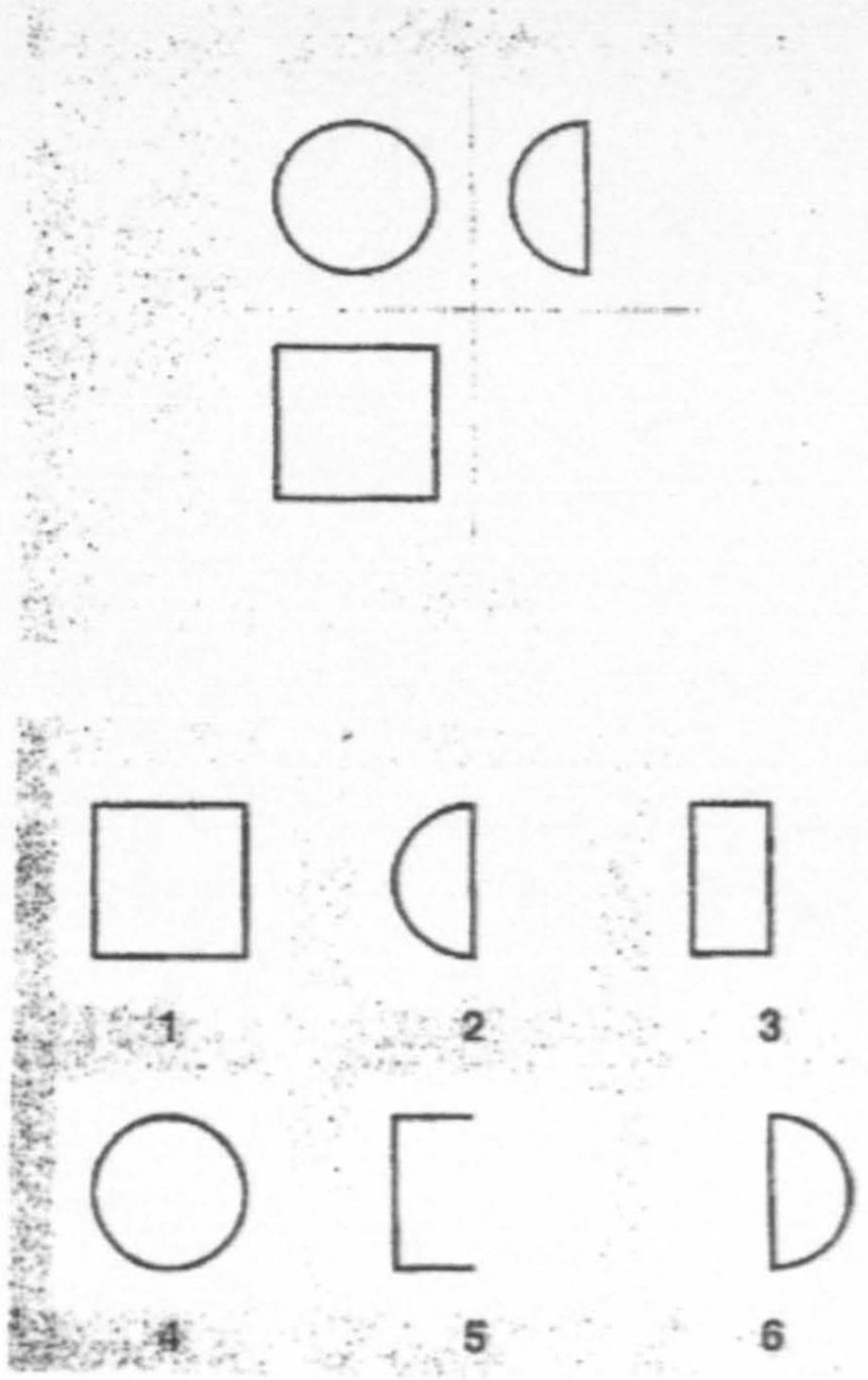
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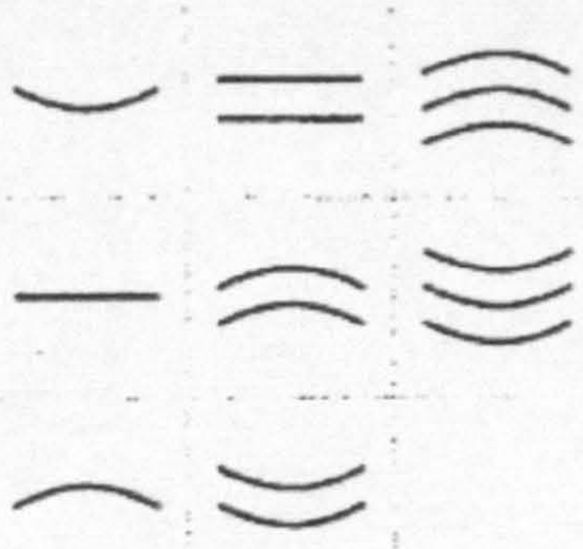

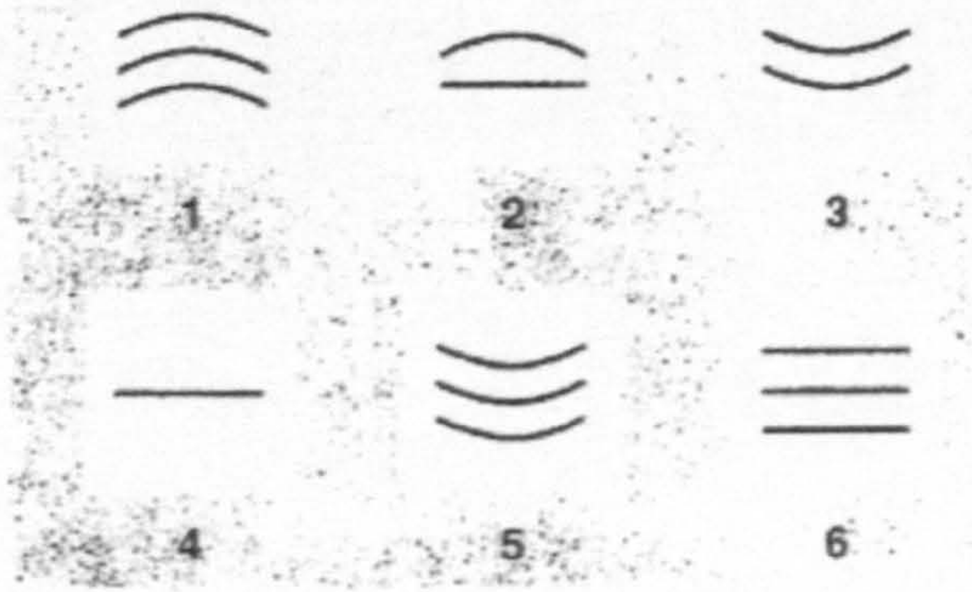
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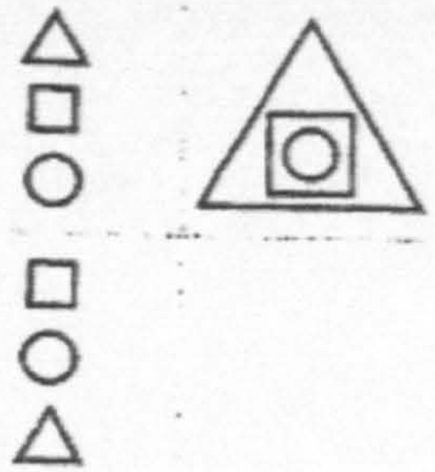

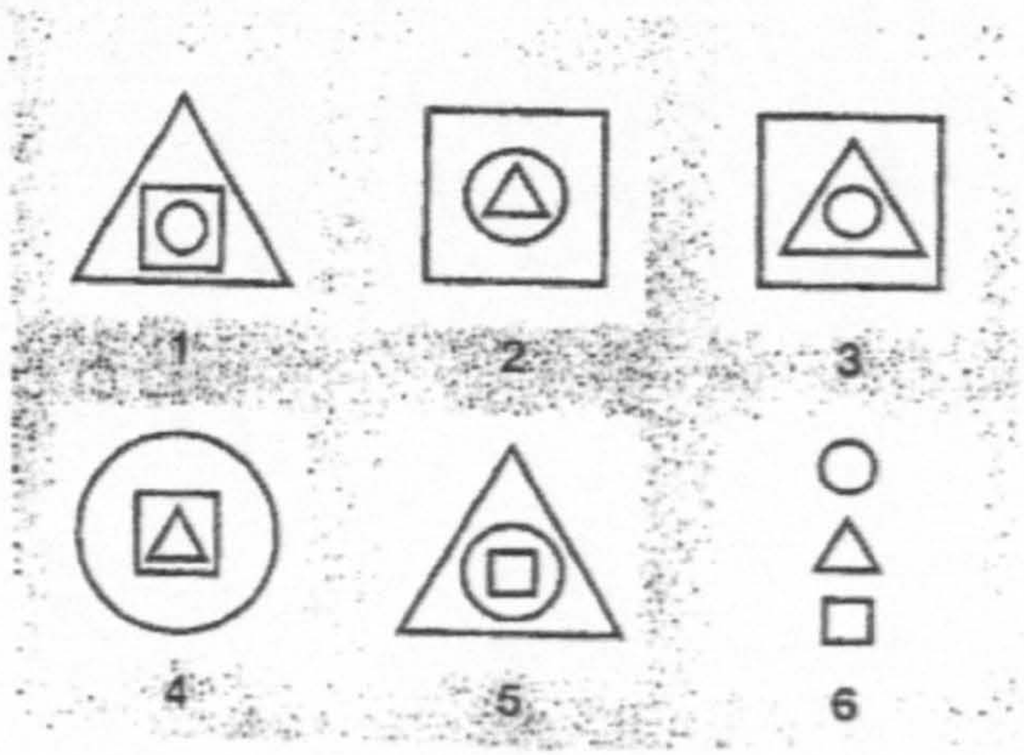
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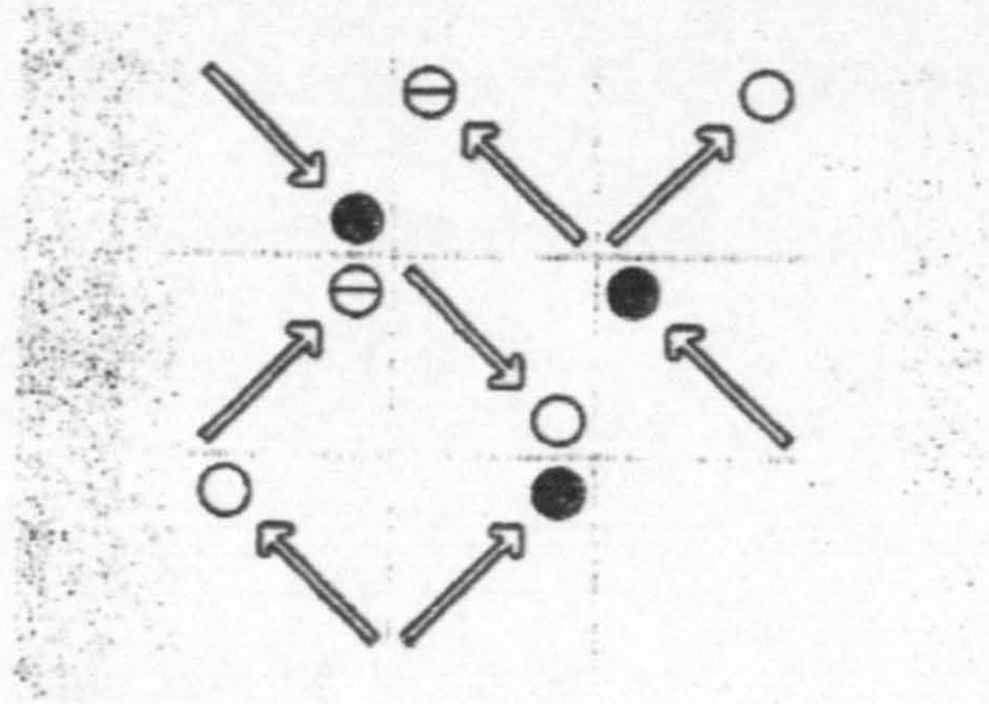

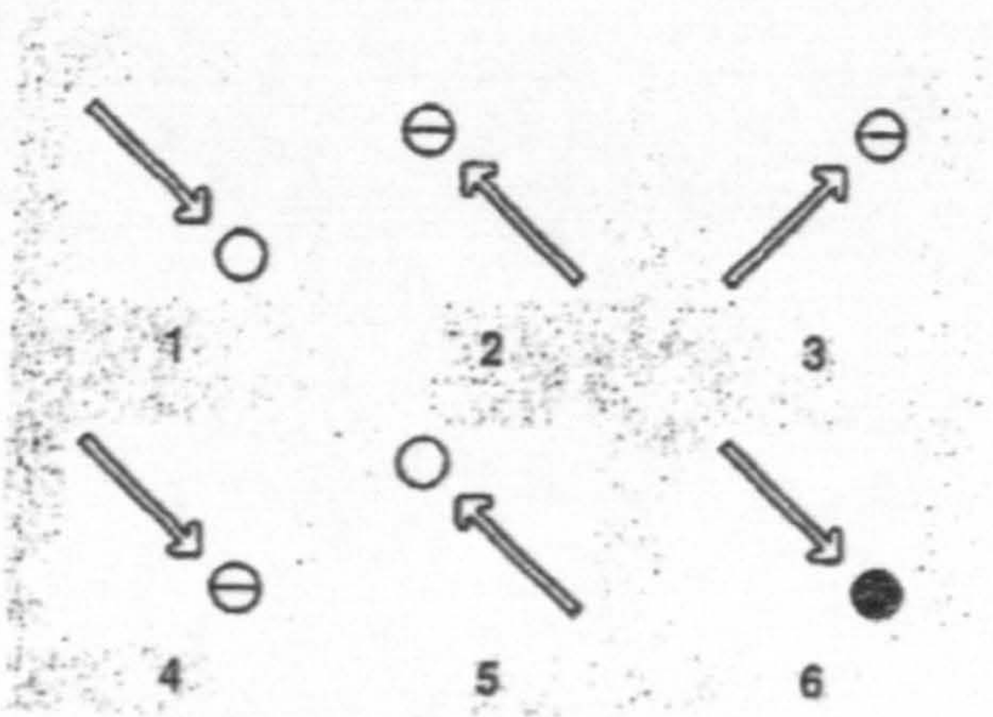
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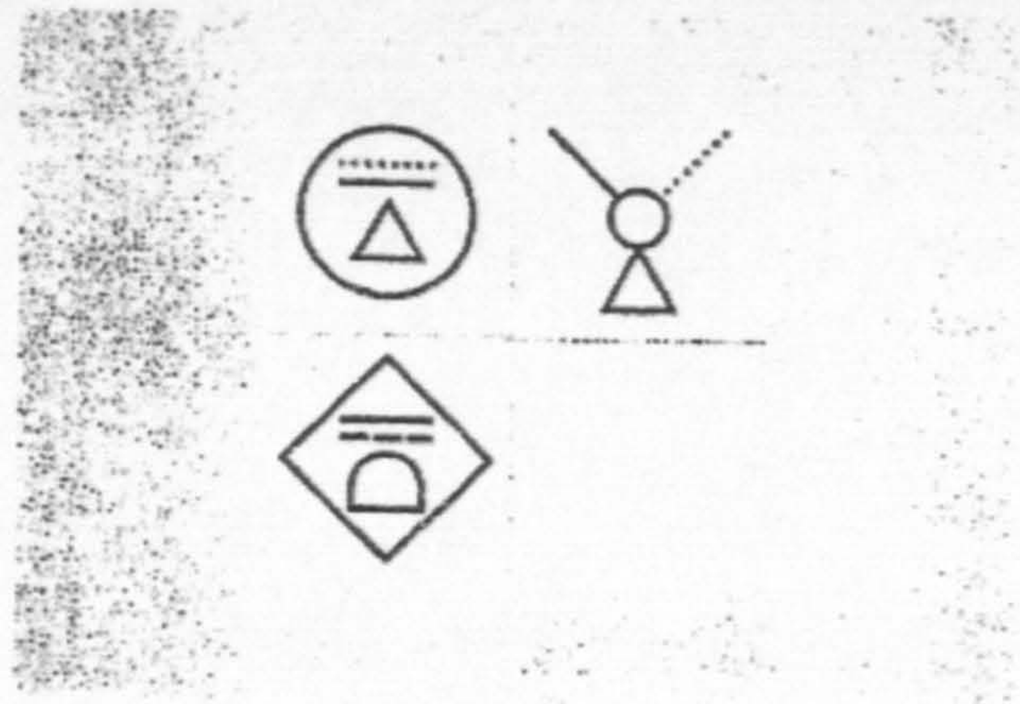

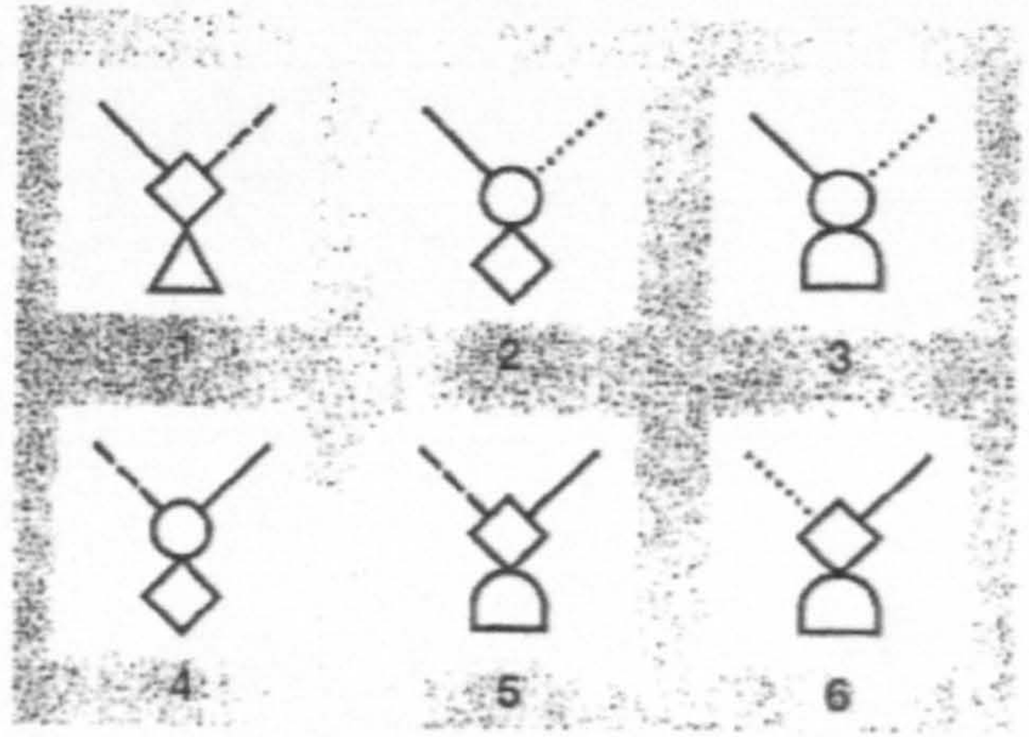
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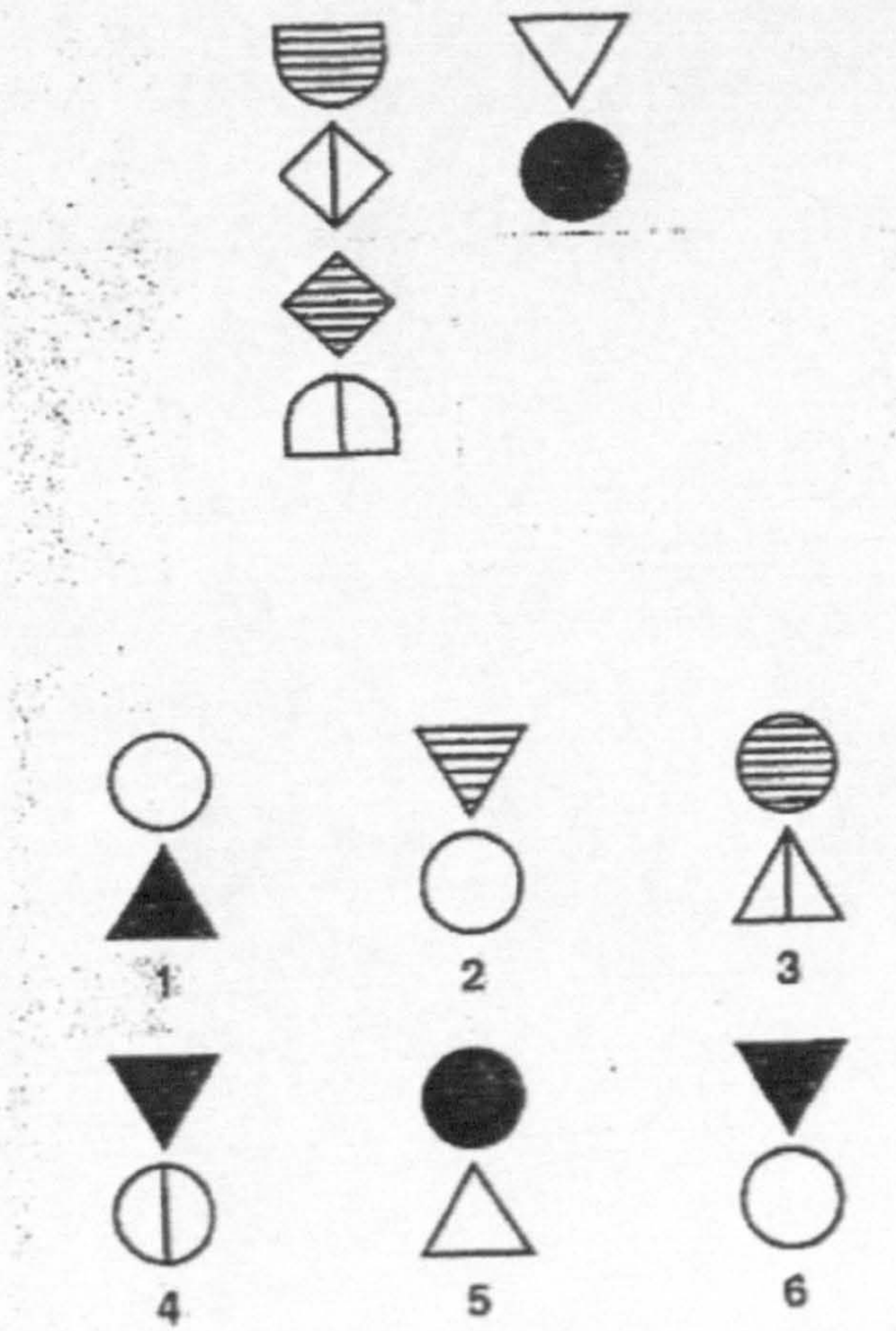
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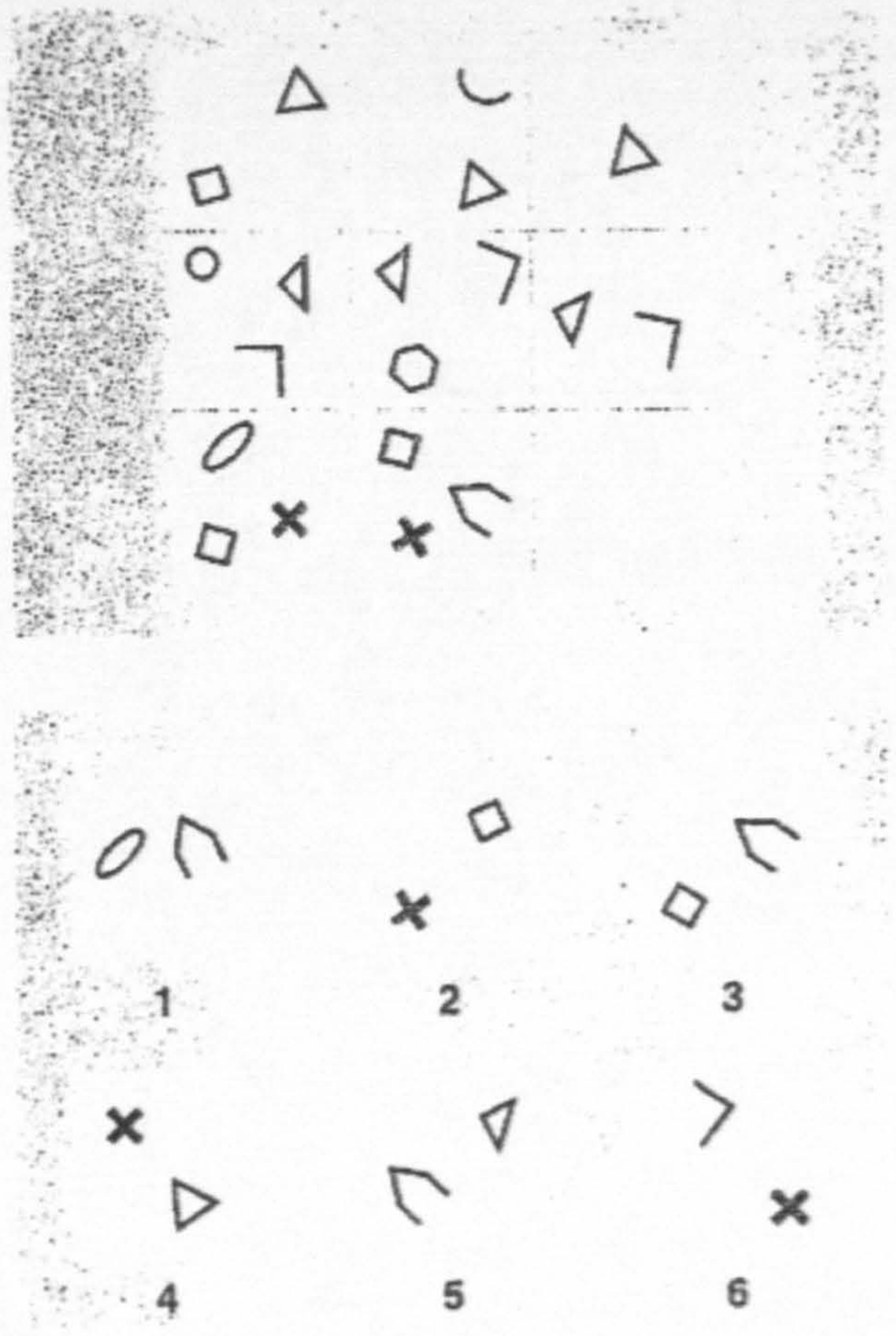
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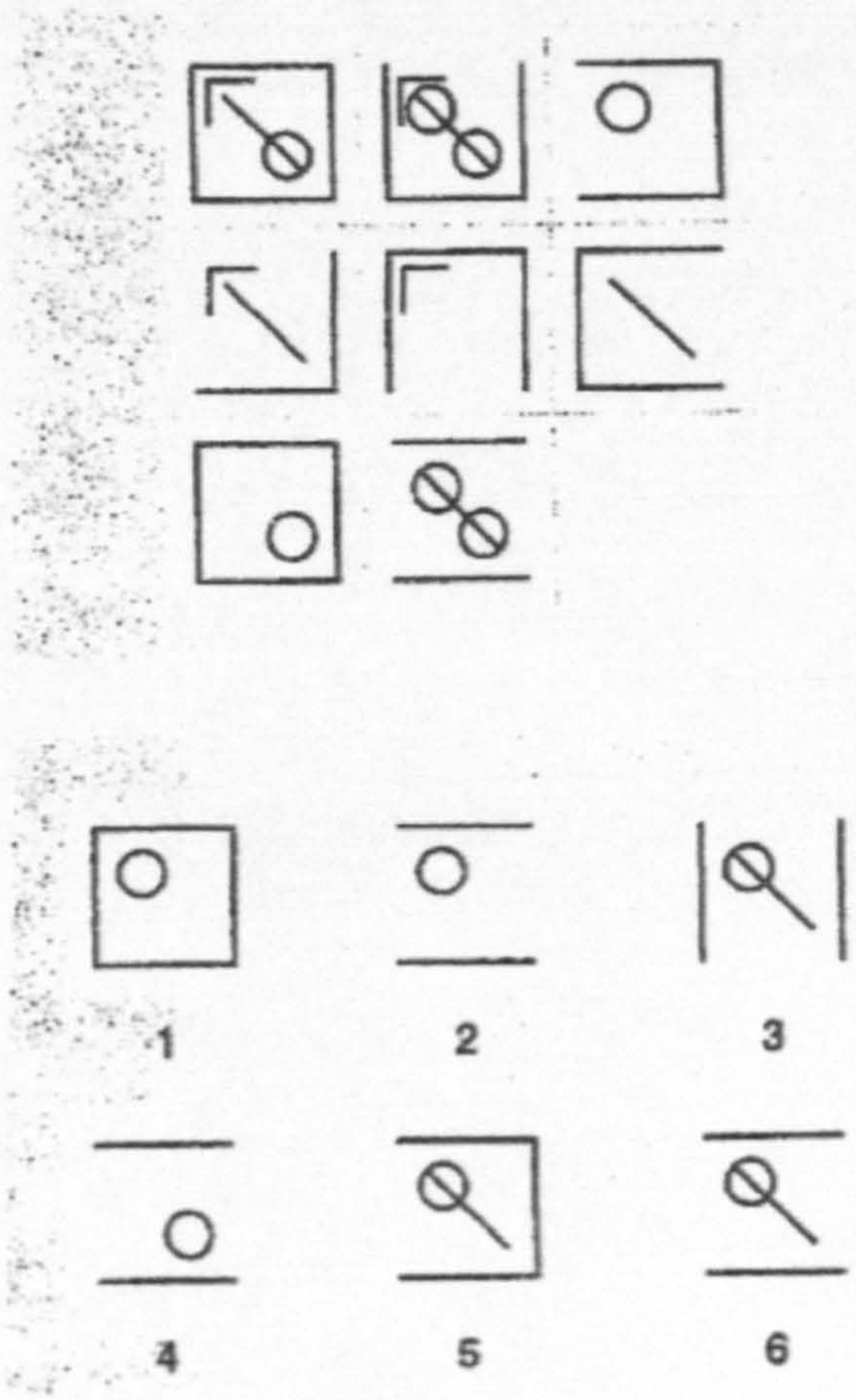


END – Do NOT turn over to the next page yet

Number search test

You will be presented with one page of numbers.
Circle blocks of THREE CONSECUTIVE ODD NUMBERS.

Find as many as you can, as quickly as you can. Move across each

row, from left to right.

You must remember **2 RULES**:
(1) Each number is only allowed in one line of circled numbers

3 11 7 4 6 5 5 13 11 1

(CORRECT) (INCORRECT)

(2) Circled numbers cannot span two lines

7 4 12 13 8 1 9 4 11 5

3 16 14 13 9 12 2 5 10 6

(INCORRECT)

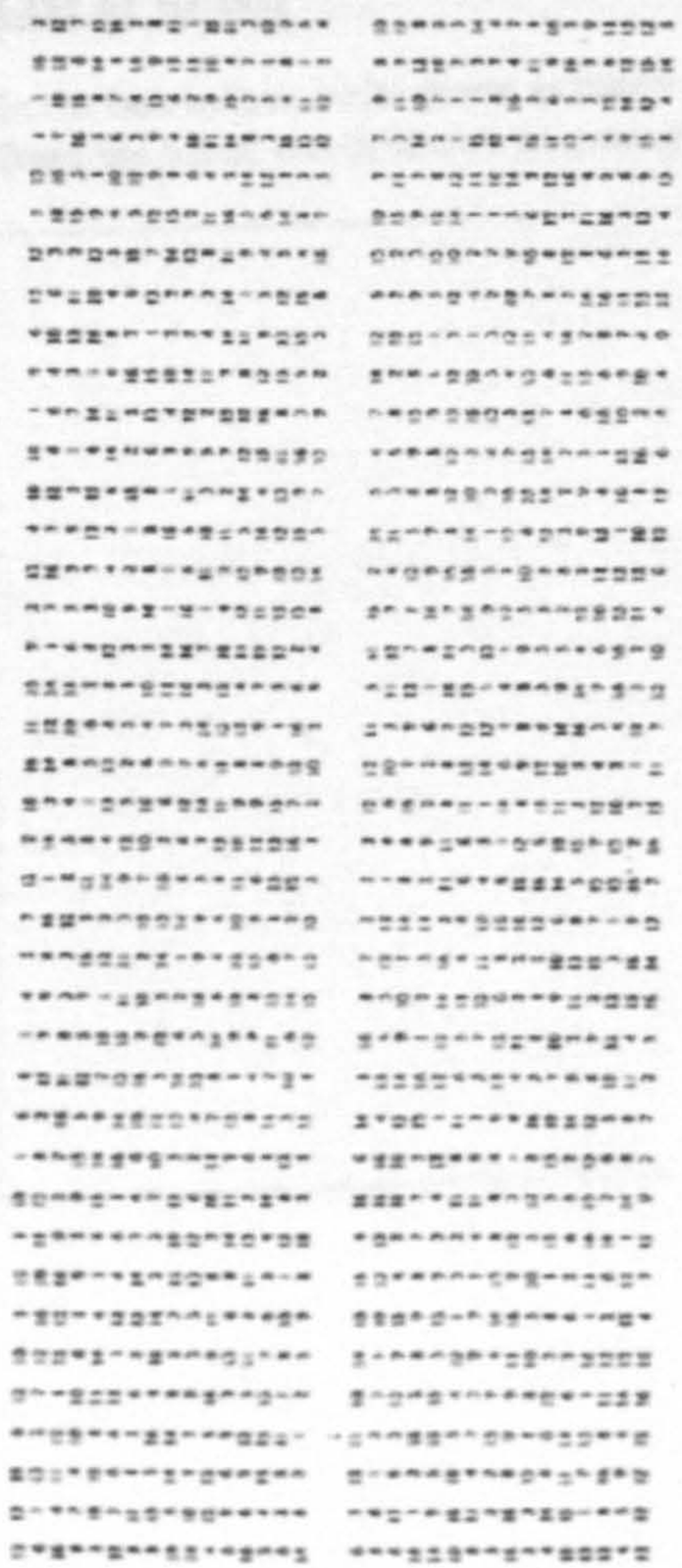
EXAMPLE

12 1 5 7 8 4 13 4 8 10

13 4 5 11 9 4 14 3 13 15

Do NOT turn over to the next page yet

Circle blocks of THREE CONSECUTIVE ODD numbers, moving across each row from left to right.
Find as many as you can, as quickly as you can.



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Subtract by seven

A number will be given to you. From this number you must count down in sevens as quickly and as accurately as you can.

You don't need to write anything down.

Do NOT turn over to the next page yet

List of words

In the box below, write down as many words as you can remember from the list of words shown to you earlier.

Do NOT turn over to the next page yet

Task Demand

Rate the tests

There are three questions for each one of the tests you did. Rate these tests, using the rating scale below each question. Write down your answer in the box provided.

(1) How easy or difficult did you find each one of the tests?

0	**=**	**very easy**
1	**=**	**easy**
2	**=**	**neither easy or difficult**
3	**=**	**difficult**
4	**=**	**very difficult**

Words beginning with the letter 'S'	☐
Remember the list of 15 words (the first time)	☐
Stroop task (the one with the colours)	☐
Find the missing shape	☐
Number search test	☐
Subtract by Seven	☐
Remember the list of 15 words (the second time)	☐

(2) How much effort did you put into doing each one of the tests?

0	**=**	**no effort at all**
1	**=**	**a little effort**
2	**=**	**some effort**
3	**=**	**quite a lot of effort**
4	**=**	**a great deal of effort**

Words beginning with the letter 'S' ☐

Remember the list of 15 words (the first time) ☐

Stroop task (the one with the colours) ☐

Find the missing shape ☐

Number search test ☐

Subtract by Seven ☐

Remember the list of 15 words (the second time) ☐

(3) How tiring did you find each one of the tests?

0	**=**	**not at all tiring**
1	**=**	**slightly tiring**
2	**=**	**moderately tiring**
3	**=**	**very tiring**
4	**=**	**extremely tiring**

Words beginning with the letter 'S' ☐

Remember the list of 15 words (the first time) ☐

Stroop task (the one with the colours) ☐

Find the missing shape ☐

Number search test ☐

Subtract in Sevens ☐

Remember the list of 15 words (the second time) ☐

Thank you for completing the tests

II.8 List of synonyms for the Mood Scales

Friendly	warm and well disposed to other people
Nervous	anxious, edgy
Drowsy	sleepy
Happy	pleased, cheerful
Calm	peaceful
Uncertain	unsure, doubtful
Sad	miserable, depressed
Energetic	lively, bouncy
Muddled	mixed-up
Relaxed	tranquil, unstressed
Dissatisfied	discontented, unhappy
Alert	attentive
Confident	sure
Tired	exhausted, worn out
Angry	irritated, crossed
Contented	satisfied
Lively	sparkling, animated
Tense	stressed, uptight
Sluggish	slow moving, listless
Clearheaded	able to think clearly
Hungry	starving, famished, need to eat
Thirsty	dry, parched, need to drink

II.9 Immediate and delayed memory task: information on the list of words used

This is the website that was used:
http://www.math.yorku.ca/SCS/Online/paivio/

Version 1	Frequency	Imagery	Concreteness	Meaning-fulness	Number of syllables	Number of letters
product	50	4.2	5.8	5.12	2	7
deed	50	3.63	4.19	5.32	1	4
hide	50	3.8	5.45	5.63	1	4
glory	50	4.13	1.77	5.88	2	5
costume	25	5.8	6.12	6.19	2	7
banner	23	5.93	6.58	6.2	2	6
gallery	28	5.97	6.49	5.88	3	7
breeze	29	5.87	5.94	7.33	1	6
tool	40	5.77	6.8	6.88	1	4
victory	50	4.93	2.95	6.12	3	7
bloom	35	5.63	5.82	5.12	1	5
angle	30	5.5	5.19	6.24	2	5
origin	48	2.3	3.25	5.32	3	6
tribute	22	3.57	3.12	5.52	2	7
vision	45	4.73	4	6.39	2	6
Total	38.33	4.78	4.90	5.94	1.87	5.73

II.10 Certificate of attendance

UNIVERSITY OF LONDON
KING'S COLLEGE

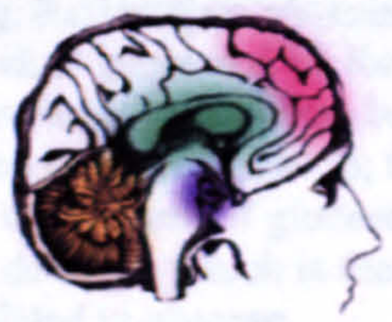
DEPARTMENT OF NUTRITION AND DIETETICS

This is to certify that

_____

took part in

The Breakfast and Learning Study



June -July 2005

II.11 Blood Glucose meters' working principle

Blood glucose meters use capillary whole blood to measure glucose either in whole blood or plasma (plasma calibrated meters), and any potential operator should be familiar with the type of the meter they are using to avoid clinical misinterpretation. The present IFCC (International Federation of Clinical Chemistry) recommendations suggest using a constant factor of 1.11 for converting glucose concentration, based on water concentrations of normal whole blood and of normal plasma (D'Orazio *et al.*, 2005). The converted result equals the concentration of glucose in plasma when the haematocrit (HCT) and water concentrations are normal. Plasma calibrated meters have made this conversion unnecessary by including an algorithm in their system, which converts whole BG concentration to plasma. The new generation of BG sensors respond to the molality of glucose (amount of glucose per unit of water mass), which is the same in whole blood and plasma, as glucose and water distribute freely between erythrocytes and plasma. Nonetheless, the concentration of glucose in these two compartments is different for a given concentration of glucose in plasma, because erythrocytes have a lower water concentration than plasma (plasma>whole blood).

Blood glucose meters' working principles are explained in detail somewhere else (Turner *et al.*, 1999), but will be briefly stated here as well. Blood glucose meters are biosensors, devices that combine a biological component with a physicochemical detector component (transducer), used to detect an analyte, glucose in our case. Blood glucose meters, as biosensors, are made up of the biological element (e.g. enzymes commonly used in the measurement of glucose include glucose oxidase, glucose dehydrogenase, and hexokinase.), the transducer, which is electrochemical, and the detector.
Biosensors for glucose are 'direct reading' as they measure glucose directly, i.e., without previous dilution of the sample. In the case of glucose meters, **electrochemical** sensors (glucose reacts with a mediator to generate electrons) could be either potentiometric (1st

generation, measure change in charge), amperometric (2nd generation, measure change in current) or coulometric (3rd generation, measure change in current, where enzyme and mediator are co-immobilized at an electrode surface). In electrochemistry the number of electrons generated by the oxidation of glucose is quantified as follows: with the application of voltage, the mediator captures the electrons generated. These electrons are transferred to and counted at the electrodes, either as charge or current. A detector converts the resulting charge or current to an electronic signal and translates that signal to glucose concentration. The amperometric sensors in comparison to the potentiometric are largely unaffected by the oxygen, uric acid, vitamin C, and acetaminophen concentration in the blood sample. Coulometric sensors convert all the glucose in a blood sample into an electrical current, rather than a small percentage of glucose (amperometric), allowing the operator to use the smallest sample size of any available technology.

The older generation of glucose meters were using **photometric** transducers (reflectance photometry), rather than electrochemical ones, which respond to changes in absorption or fluorescence (measure a coloured product) (Lifescan, 1998). Using an enzyme as a catalyst, glucose reacts with another compound to generate a coloured product. The amount of coloured product is directly proportional to the amount of glucose in the blood. These meters require removal of excess blood by wiping. Reflectance photometry quantifies the intensity of the coloured product generated by the enzymatic reaction. Light of a specific wavelength which can be absorbed by the coloured product is emitted onto the test strip; the light reflected is inversely proportional to the amount of glucose in blood. The reflected light is captured by a detector, which is then converted to an electronic signal and translated to glucose concentration.

III Predicting glycaemic and insulinaemic responses from mixed breakfast meals

III.1 Administration protocol

(While on of us is going through the administration protocol with the subject the other one can set up the breakfast meals, which will have been prepared from the day before)

2 participants will be seen each day, with a 10-15 min interval

1. Write down the time of arrival

2. Introduce yourself and make sure they have understood what the study involves by briefly explaining it to them.

3. Give them the consent form and ask them to sign it (only on the first appointment for each participant).

4. Ask the questions from the researcher's booklet, and decide whether to continue or not

5. Measure height (only on the first appointment for each participant) and weight
Take 2 measurements, and if different take a 3rd one
Ask the participant to take off their shoes, their jumper/ jacket and empty their pockets (If they are wearing any heavy clothing, jacket or jumper).

6. Take one fasting finger prick blood sample, but make 2 measurements for glucose (for each one of the meters); if different take a 3rd one as well.
RECORD TIME
Measure glucose using both glucose meters
(in this order: AVIVA - whole blood, FREESTYLE MINI - plasma calibrated)
Measure haemoglobin, using HEAMOCUE 201+

Exclude if capillary,
wholeblood glucose: ≥ 100 mg/dl, and < 110 mg/dl
(5.6 mmol/L) (6.1 mmol/L)
AVIVA
plasma glucose: ≥ 110 mg/dl, and < 126 mg/dl
(6.1 mmol/L) (7.0 mmol/L)
FREESTYLE MINI

7. *Cannulate the subject.*

8. Take fasting venous blood sample (11 ml of blood); for the rest of the venous samples take only 9 ml.
RECORD TIME

9. Separate the blood into the 3 different vacutainers, as follows
- 6 ml for the serum tube (red lid)
- 2 ml in the EDTA tube (levander lid): ONLY FASTING SAMPLE
- 3 ml in the fluoride oxalate (grey lid)

* (see lab method on next page)

10. *Take the participant in the dinning room.*

11. *Instruct the participant to consume the breakfast meal at a comfortable pace within 15 min. They can't eat or drink or eat anything else after that.*
RECORD TIME

12. Time zero will be regarded as the time when eating commenced (when they put the food or drink in their mouth).

Record time and start counting using a stopwatch

13. Blood samples will be collected at 15, 30, 45, 60, 90, 120, 150 and 180 min after time zero.

14. 2-3 min before the next blood sample take the participant to the bleeding room.

15. Each time take the finger prick first and then the venous blood sample. You should do that as quicly as possible (immediately after, or if the subject doesn't object at the same time). Always confirm with the participant that they are feeling

allright. If the feel faint exclude them from the study, and give them some orange juice and something to eat as well.

16. For Haemoglobin, apart from the fasting sample, take 2 more measurements, preferably at 60 min and then at 180 min.

17. After taking the blood sample take the participant back to the dining room.

18. They are allowed to read magazines etc. They are not allowed to leave the metabolic room, unless they want to go to the toilet, in which case it is advised to go with them, in case they feel faint. Tell them that it is important to keep physical activity to a minimum during the testing; and to be as calm and relaxed as possible. Ideally, they should remain sitted the whole time, until it is time to take the next blood sample.

19. After the last blood sample, take the participant back to dinning room. Thank them for the participation. Give them a sandwich and some orange juice. Make the appointment for the next time, and give them the instruction sheet. Remind them to have the same dinner, as stated in their instruction sheet.

20. Make a phonecall to the courier company. Sent the EDTA tube to Dr Sherwood.

21. Sent the cryovial boxes with an excel sreadsheet (id, time, breakfast meal).

LAB METHOD

1. Mix the tubes
2. Put the EDTA (levander) tubes in ice box.
3. Let the serum tube (red lid) stand for 15 - 20 min, until it clots.
4. Centrifuge the serum tube and the fluoride oxalate tube for 15 - 20 min (balance them out, 2 - 4 degrees C, 3 rph).
5. Mark the cryovials with a marker: ID number, time (0, 15, 30, 45, 60, 90, 120, 150, 180 min), and breakfast meal (1, 2, 3, 4, 5).
6. Separate the serum from the serum tube (red lid) and the plasma from the fluoride oxalate tube (grey lid). Be extra careful.
7. Place half of the serum in 1 ml aliquot, and the the rest in another 1 ml aliquot (cryovial). If more than 1 ml per cryovial, use the 2 ml ones

8. Place the plasma in a 2 ml cryovial.
9. Use different cryovial boxes for each one of the 3 aliquots (plasma, serum, serum).
10. Put them in the freezer in -20 °C, and store them for less than a month.
11. Keep everything clean and tidy.

Serum/ plasma difference
Serum is the liquid part of blood AFTER coagulation, therfore devoid of clotting factors as fibrinogen. plasma is the liquid, cell-free (by centrifugation, for example) part of blood, that has been treated with anti-coagulants
Serum= plasma - fibrinogen.
To get serum, you need to let the blood clotted and after centrifuge. The upper supernatant is the serum without any clotting factors.
To get plasma, you add anticoagulant like heparin in the blood to avoid clotting and then centrifuge. The upper supernatant is the plasma which contain clotting factors (fibrinogen). Because of the anticoagulant these clotting factors cannot work and because of their density, they cannot go to the bottom after centrifugation. That`s why they are mixed in the upper liquid which called plasma.
Plasma and serum are both fluid components of the blood. However, serum is only present in the blood subsequent to clotting, after an injury. In essence, serum is identical to plasma, but it does not contain the components necessary for the clotting reaction (such as fibrinogens).

Plasma comprises approximately 55% (the other 45% is red blood cells) of the total blood volume, and contains salts and ions such as calcium, sodium, potassium, and bicarbonate. It also contains larger molecules such as amino acids, lipids (fats), vitamins, and hormones. Approximately 91-92% of blood plasma is water, 7-8% is protein, and 1-2% is other solutes, such as electrolytes and nutrients.

III.2 Circular e-mail

'Circular email for use for recruitment of volunteers for study ref 05-06-25, approved by King's College London Research Ethics Committee (REC). This project contributes to the College's role in conducting research, and teaching research methods. You are under no obligation to reply to this email, however if you choose to, participation in this research is voluntary and you may withdraw at anytime.'

BREAKFAST AND BLOOD GLUCOSE LEVELS

We have recently found that breakfast can influence how well people do on tests of learning ability (cognitive function) two hours after eating. The effect is due in part to the ability of different types of meal to raise blood glucose levels (glycemic potency). We would like to learn more about the way in which different types of meals at breakfast influence blood glucose response in the hours after eating. We would like to invite you to take part in a study to clarify how four types of breakfast meal affect blood glucose levels. We will also measure your iron status, and tell you whether you are anaemic or not.

We are inviting healthy young women and men aged 18–25 years old to take part. People with diabetes, anaemia or other serious illnesses, and those who are taking medicine that affects glucose metabolism are not eligible. Pregnant or lactating women and elite athletes also should not take part.

If you are interested in taking part, you will be asked to do the following:

1. Complete a short screening questionnaire, which includes questions about your medical history and dietary habits.
2. Make four visits to the Department of Nutrition and Dietetics (room 4.26, corridor B), 4th Floor, Franklin Wilkins Building, Waterloo campus.
3. Have a dinner of your choice the evening before the appointment (before 9pm), and nothing else until your appointment (you can have water).
4. Eat one of four different breakfasts that will be provided at each visit on the morning of your appointment. The breakfast meals typically contain cereal, milk, juice, bread, and sometimes a meat product (e.g. sausage).
5. Provide finger prick and venous blood samples. These will be collected when you arrive and then again 15, 30, 45, 60, 90, 120, 150 and 180 minutes after breakfast. The insertion of a small needle into a vein of the forearm for blood taking may cause slight discomfort and bruising; the needle will be placed only once on each appointment, and from that multiple small samples will be drawn.
6. During that time you can watch TV or videos or read books or magazines.

You will be reimbursed for your time.

The study has been approved by the King's College Research Ethics Committee (05/06-25) and will take place between February 2006 and March 2006.

If you are interested in participating in this study or would like further information, please contact me by e-mail at eirini.michas@kcl.ac.uk or telephone 020 7848 4594/ 07859081757.

Thank you very much,
Renata

III.3 Information sheet

INFORMATION SHEET FOR PARTICIPANTS

GLYCEMIC POTENCY OF COMPOSITE BREAKFAST MEALS

We would like to invite you to participate in the PhD research project looking into the glycemic potency (ability to raise blood glucose levels) of composite breakfast meals. You should only participate if you want to; choosing not to take part will not disadvantage you in any way. Before you decide whether you want to take part, it is important for you to understand why the research is being done and what your participation will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. If you do decide to take part, please let us know beforehand if you have been involved in any other study during the last year.

Why are we carrying out the research?
The proposed study is part of a larger study looking into the glycemic potency of breakfast and cognitive function. In order for the role of the glycemic potency of breakfast on cognitive function to be more clearly evaluated, a better understanding of the physiological properties of mixed meals needs to be established; this can be done by measuring the blood glucose levels over a period of three hours for a variety of mixed meals.

Who will take part in the study?
All students aged 18-25 in King's College London will be asked to take part. Not all students who consent will be participating, but some who will be selected at random. Students who have any of the following will not be selected: anaemia, thalassaemia, sickle cell anaemia, other causes of anaemia (hookworm infection, schistostomiase and malaria), diabetes or other disorders of glucose metabolism, or other acute or chronic illnesses or diseases, learning disabilities. Elite athletes and students with Body Mass Index (BMI = kg/m²) of less than 20 and more than 25 will not be selected as well.

What will you be asked to do?
If you agree to take part you will be asked to complete a short questionnaire, which will include questions about about your age, height, weight, gender, medical conditions, breakfast habits. You will be measured for height and weight and will be asked to be seen on four separate occasions, one week apart, at the Department of Nutrition and Dietetics (Waterloo campus); on each occasion you will receive a different breakfast meal. On the day before each appointment you will be asked to consume a dinner of your choice at a certain time, which has to be the same before each test; you will be instructed not to have anything else apart from that until your appointment next day (with the exception of water); you will also be asked to avoid any strenuous physical exercise. On the day of your appointment you will be measured for weight and height; finger prick and venous blood samples will be collected at the time of your arrival and then again 15, 30, 45, 60, 90, 120, 150 and 180 minutes after breakfast. As far as the venous blood samples are concerned, a blood taking needle will be placed on your brachial vein, which means that you will be punctured only once. We will measure your glucose and iron levels. We will be able to tell you whether you are at risk of or you have anaemia. There is no risk of infection when the blood samples are taken, as only sterilised equipment and disposable lancets will be used. You might experience minimal discomfort associated with giving blood, and/ or minor bruising caused by venous blood sampling. The whole procedure will take approximately three hours, during which you will be able to watch TV/ videos or read magazines. You will be compensated for you time with a small token of appreciation; on completion of the study you will receive £50.

Who will see this information?
Only the people from King's College London who are directly involved in the research will see the information for individual students. The information collected will not be shown to anyone else. All the information collected will be kept strictly confidential. You will not be identifiable in any verbal or written reports about the research. At the end of the study you will be informed about your iron status.

This study has been approved by the King's College Research Ethics Committee, ref no: 05/06-25

INFORMATION SHEET FOR PARTICIPANTS

When will the work be carried out?
The study will be carried out between January and February 2006 by Ms Renata Miche at King's College London. If you have any questions about the study, please ring 020 7848 4549.

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. You may freely withdraw at any time and without giving a reason, even when consent has already been given. A decision to withdraw at any time, or a decision not to take part, will not affect you in any way. In the event of you suffering any adverse effects as a consequence of your participation in this study, you will be compensated through King's College London's 'No Fault Compensation Scheme'

For further information please contact:
Dr Michael Nelson
Reader in Public Health Nutrition
Department of Nutrition and Dietetics
King's College London
Franklin-Wilkins Building
150 Stamford Street
London SE1 9NH
tel: 020 7848 4349
e-mail: michael.nelson@kcl.ac.uk

Renata Miche
PhD Student
Department of Nutrition and Dietetics
King's College London
Franklin-Wilkins Building
150 Stamford Street
London SE1 9NH
tel: 020 7848 4594
e-mail: emmi.miche@kcl.ac.uk

This study has been approved by the King's College Research Ethics Committee, ref no: 05/06-25

III.4 Screening form

Please contact me if any of the questions are unclear.

Gender: Male/Female (please circle)

Date of Birth: _____(day/month/year)

Contact details (phone number): _____(day)

_____(evening)

_____(e—mail) _____(mobile)

1. Do you have or is there a family history of any of the following conditions?

(the conditions listed here are the exclusion criteria for the study)

Please tick (√) one box or two boxes in every row

	Yes, I do have the condition	Yes, family history of condition	Neither
Sickle-cell anaemia			
Haemophilia			
Thalassaemia traits			
Any other blood disorder (please specify)			
Diabetes			
Any other glucose tolerance dis (please specify)			
Any other chronic disease (please specify)			

2. Do you have any learning disabilities?

Please tick (√) ONE box

Yes ☐

No ☐

If YES, please give details:

_____

_____

_____

_____

3. Do you take any medication prescribed by a doctor on a regular basis?

Please tick (√) ONE box

Yes ☐

No ☐

If YES:
Please write down what type:

What type

4. Are you on a special diet for medical reasons?

Please tick (√) ONE box

Yes ☐

No ☐

If YES, please describe this diet and say why you are on it:

_____

_____

_____

_____

5. In the past four weeks, have you had any infections?

Please tick (√) ONE box

Yes ☐

No ☐

If YES, please give details:

Type of infection	Why	How long did it last?

6. Are you taking vitamin or mineral tablets/ supplements now?

Please tick (√) ONE box

Yes ☐

No ☐

If YES: Please tell me what type of supplements you take and how often you take them:

Type	How often and Time of the Day

7. Do you follow a special diet for religious reasons?

Please tick (√) ONE box

Yes ☐

No ☐

If YES, please give details:

_____

_____

_____

_____

8. Are you currently on a diet?

Please tick (√) ONE box

Yes ☐

No ☐

If YES:
Are you trying to lose OR gain weight?

Please tick (√) ONE box

Lose ☐

Gain ☐

If YES, please give details (how long, by a dietician/ personal diet, types of food avoided or excluded, weight lost/gained in the last 6 months):

_____

_____

_____

9. How would you describe your body weight?

Please tick (√) ONE box

Very underweight ☐

Slightly underweight ☐

About right ☐

Slightly overweight ☐

Very overweight ☐

10. Do you have any food intolerances/ allergies?

Please tick (√) ONE box

Yes ☐

No ☐

If YES, please give details:

_____

_____

_____

11. **Are you a vegetarian?**

Please tick (√) ONE box

Yes ☐

No ☐

If YES, please give details (name the types of food you don't eat):

_____

_____

_____

12. **How often do you have breakfast?**

Please tick (√) ONE box

Never ☐

Once a week ☐

Twice or more a week ☐

Every day ☐

Other, please state _____ ☐

If your answer is NEVER, please go directly to question number 14.

13. **If you do have breakfast (even if not every day), please answer the following questions:**

a. **What do you typically have for breakfast?**

FOOD/BEVERAGE	AMOUNT

b. **Please write down the time that you have breakfast:**

time you start eating breakfast: _____ (AM)

time you finish eating breakfast: _____ (AM)

14. a. **What do you typically have for dinner?**

FOOD/BEVERAGE	AMOUNT

b. **Please write down the time that you have dinner:**

time you start eating dinner: _____ (AM)

time you finish eating dinner: _____ (AM)

15. **Please write down your current weight.**

stones: _____ **pounds:** _____ **OR kilos:** _____

16. **Please write down your height.**

cm: _____ **feet:** _____ **OR** **inches:** _____

17. **What time do you typically go to sleep every night, and what time do you typically wake up in the mornings?**

a. **time you sleep** _____ PM

b. **time you wake up** _____ AM

18. **Which of the following do you think best describes your ethnic origin?**

Please tick (√) ONE box

White Caucasian		Pakistani	
Black-Caribbean		Bangladeshi	
Black-African		Chinese	
Black-Other		Asian-Other	
Indian-Other		Other, please specify	

19. Do you exercise?

Please tick (√) ONE box

Never	☐
Once a week	☐
Twice or more a week	☐
Every day	☐
Other, please state _____	☐

If you DO exercise, please give details:

Type of exercise	How long per time	Intensity

20. Do you smoke?

Please tick (√) ONE box

Yes	☐
No	☐

If YES, please give details:

How often	Cigarettes/ day

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE

ALL INFORMATION IS CONFIDENTIAL, FOR RESEARCH PURPOSES ONLY

III.5 Consent form

☐☐☐☐☐

GLYCAEMIC POTENCY OF COMPOSITE BREAKFAST MEALS

Thank you for considering to take part in this research. The project will be explained to you before you agree to take part.

If you have any questions arising from the Information Sheet or explanation already given to you, please ask the researcher before you decide whether to join in. You will be given a copy of this Consent Form to keep and refer to at any time.

I understand that if I decide at any other time during the research that I no longer wish to participate in this project, I can notify the researchers involved and be withdrawn from it immediately.

I consent to the processing of my personal information for the purposes of this research study. I understand that such information will be treated as strictly confidential and handled in accordance with the provisions of the Data Protection Act 1998.

Participant's Statement:

I _____

agree that the research project named above has been explained to me to my satisfaction and I agree to take part in the study. I have read both the notes written above and the Information Sheet about the project, and understand what the research study involves.

Signed _____ **Date** _____

Investigator's Statement:

I _____

confirm that I have carefully explained the nature, demands and any foreseeable risks (where applicable) of the proposed research to the volunteer.

Signed _____ **Date** _____

III.6 Instructions sheet

Breakfast meals and blood glucose levels

Your Appointments are on:

_____ _____ _____ _____ _____

8:00 am
4th Floor, Corridor B (extension number 4594)
Department of Nutrition and Dietetics
King's College London
150 Stamford Street London SE1 9NH
nearest tube: Waterloo station

On the day before each appointment, please:

- Eat your normal diet.
- Avoid alcohol consumption.
- Avoid smoking.
- Restrict the consumption of caffeine containing drinks (eg coffee, tea, cola drinks).
- Restrict your participation in intense physical activity (eg long periods at the gym, excessive swimming, running, aerobics)
- Have a dinner of your choice, but this must be the same meal before each appointment. Please write down what you ate and the quantity and bring this information with you *(see page 3)*. Your dinner should be finished by 9:00 pm.
- Consume dinner in ≤ 20 min, and sit quietly after dinner and before bed.
- Don't eat or drink anything else after 9 pm. You can have water, but in moderation.
- Have a good night's sleep (~ 8 hours).

On the morning of the study, please:

- Complete page 3 of this sheet and bring it with you to your appointment.
- Don't eat or drink anything. You can have water in moderation, as long as it is 45 min before your appointment.
- Avoid any form of physical activity; please, come to the department using the least strenuous means of transport.
- Bring something to read if you wish. We will have magazines/ tv available.
- Come to the department of Nutrition and Dietetics at 8:00 am. You will meet either Renata outside corridor B.

If you have any questions, please feel free to contact us at any time:

Renata Micha
E-mail: eirini.micha@kcl.ac.uk
Tel: 07859001757

DINNER

Time started to eat dinner	AM
Time finished eating dinner	AM

Please, write down what you had.

FOOD/BEVERAGE	AMOUNT

III.7 Researcher's booklet

Today's date: _____ (Day/ Month/ Year)

Time on arrival: _____ AM

Gender: Male/ Female (please circle)

Breakfast meal: _____ (number)

- Did you have anything to eat and drink since you woke up this morning?

  Yes/ No

- If YES, please tell me what you had; exclude them accordingly

Probe: if water, how many glasses and when?; tea, coffee anything

_____

- Check all the other question on their instructions sheet *(see next page)*

- Did you follow the instructions that were given to you? Yes/No

- Did you have tea, coffee, coke, red bull or an alcoholic drink yesterday?

  Please tick (√) ONE box

  Yes ☐

  No ☐

If YES:
Please tell me how much and what time:

Description of drink	Time

- What was the last thing you had to drink/eat last night?

Probe: ask them to give you the instruction sheet, if not already
Last meal (food/ drink) should be by 9 pm

Time started to eat dinner	PM
Time finished eating dinner	PM

FOOD/BEVERAGE	AMOUNT

- Did you smoke yesterday or today? Yes/ No

  Yesterday/ Today

If YES, how many cigarettes? _____

- Did you have any form of physical exercise yesterday? Yes/No

  If YES, please tell me:

  a. what time was that? _____

  b. what type of exercise? _____

  _____

  _____

  c. for how long? _____

- Did you have any form of physical exercise today? Yes/No

probe: how they got to the department, walking, running etc

  If YES, please tell me:

  a. what time was that? _____

  b. what type of exercise? _____

  _____

  _____

  _____

  c. for how long? _____

- What time did you go to sleep last night, and what time did you woke up this morning?

  a. time you slept _____

  b. time you woke up _____

HEIGHT AND WEIGHT MEASUREMENTS

HEIGHT: 1)_____, 2)_____ 3)_____CM

WEIGHT: 1)_____, 2)_____ 3)_____KG

BIOELECTRICAL EMPEDANCE: _____
_____
_____

TIME NOW: _____ **AM**

Cannulate subject, take fasting finger prick and venous blood sample

BREAKFAST – TIME ZERO

Start eating: _____ **AM**
Finish eating: _____ **AM**
Total time: _____ **min** *(time with stopwatch)*

BLOOD MEASUREMENTS

TIME min	RECORD TIME		BLOOD MEASUREMENTS			
	Finger prick	Venous	AVIVA	FREE STYLE	Hb	
			Capillary	Capillary	Capillary	venous
Fasting AM	AM	AM			↑	↑
15	Min	Min				
30	min	min				
45	min	min				
60	min	min			↑	↑
90	min	min				
120	min	min				
150	min	min			↑	↑
180	PM	PM				

III.8 Macronutrient composition (%) of the breakfast meals administered

	BREAKFAST MEALS				
	HIGH GL			LOW GL	
	Low GI (M1)	High GI (M2a)	High GI (M2b)	Low GI (M3)	High GI (M4)
% Protein	11.8	12.1	11.9	17.8	17.4
% Fat	13.6	10.3	10.2	20.4	16.6
• of which saturates %	5.1	6.6	6.5	9.7	11.0
% Total carbohydrate	69.2	72.1	72.3	57.6	61.5
• of which sugar (%)	43.7	36.8	38.9	31.8	30.5
• of which starch (%)	25.5	35.3	33.5	25.8	31.0
Sugar breakdown					
• NMES (%)	29.5	22.0	24.4	8.9	7.8
• Intrinsic & Milk Sugar (%)	14.1	14.7	14.5	22.9	22.7
Sugar breakdown					
• Glucose (%)	7.8	4.7	5.1	2.1	0.4
• Fructose (%)	16.2	10.7	11.7	2.4	0.4
• Sucrose (%)	8.0	7.7	8.6	6.7	7.8
• Maltose (%)	0.0	0.0	0.0	0.0	0.0
• Lactose (%)	9.4	12.1	12.0	18.1	20.4
• Other Sugars (Oligos) (%)	2.5	1.8	1.7	2.5	1.6
% NSP	2.1	0.7	0.7	2.1	0.6

III.9 Analysis per 100g of the foods that constituted the breakfast meals

	Alpen Muesli-no added sugar (Weetabix)	Kellog's Corn Flakes	Tesco Semi-skimmed milk	Tesco Apple juice	Sugar white
Energy (kcal)	353.0	372.0	48.0	46.0	400.0
Protein	10.7	7.0	3.3	0.1	0.0
Fat	5.9	0.9	1.6	0.0	0.0
unturates	8.7	0.2	1.1	0.0	0.0
Total CHO (g)	64.3	84.0	5.0	11.1	100.0
of which sugar (g)	15.9	8.0	5.0	11.1	100.0
of which starch (g)	48.4	76.0	0.0	0.0	0.0
NMES (g)	4.2	2.4	0.0	11.1	100.0
Intrinsic & Milk Sugar (g)	11.7	5.6	5.0	0.0	0.0
Glucose (g)	4.0	1.0	0.0	2.9	0.0
Fructose (g)	4.5	0.9	0.0	7.1	0.0
Sucrose (g)	0.0	2.4	0.0	1.2	100.0
Maltose (g)	0.0	0.0	0.0	0.0	0.0
Lactose (g)	2.8	0.0	5.0	0.0	0.0
Other Sugars (Oligos) (g)	4.7	3.8	0.0	0.0	0.0
Fibre	7.7	3.0	0.0	0.0	0.0
GI	55±10	81±3	25±6	40±1	68±5
	55	81	25	40	68
GI comment	International Table	International Table	UK values	International Table	International Table

III.10 Biochemical assays for the measurement of blood glucose, insulin, cortisol, SF, SfTR and FBC

❖ ***Blood glucose***

The Bayer Advia method used for the measurement of glucose uses an endpoint enzymatic reaction. Glucose is measured using the enzymes glucose oxidase and peroxidase. In the first step glucose is converted to gluconic acid and hydrogen peroxide. The hydrogen peroxide then reacts, in the presence of peroxidase, 4-aminophenazone and phenol to produce a red quinoneimine dye. The absorbance is measured at 596/605 nm. Glucose reagent were supplied by Bayer Diagnostics Europe Ltd.

Each sample was assayed 2 times per run, 2 runs per day, for at least 10 days. Precision estimates were computed by the manufacturer according to CLSI document EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline.

	Within-Run		Total	
Level (mg/L)	SD	CV (%)	SD	CV (%)
4.4	0.02	0.6	0.07	1.6
16.3	0.08	0.5	0.19	1.2

The ADVIA glucose oxidase method is traceable to the CDC Reference Method (Neese *et al.*, 1976), which uses reference materials from the National Institute of Standards and Technology (NIST), via patient sample correlation and verified with NIST Reference serum. Assigned values of Bayer Chemistry Calibrator, Bayer Assayed Chemistry Controls, and ADVIA Chemistry Urine Controls are traceable to this standardization.

❖ ***Insulin***

The assay used to measure insulin is a solid-phase, two-site chemiluminescence immunoassay (Sandwich assay) for the measurement of Insulin in human serum. It utilises one murine monoclonal antibody, which is bound to a bead, and one rabbit polyclonal antibody conjugated to alkaline phosphatase in the form of a reagent. The Immulite 1000 analyser (Siemens Medical Diagnostic Solutions, Gwynedd, UK) was used to carry out the analysis, and the insulin was supplied by DPC, Glyn Rhonwy, Llanberis, Gwynedd, LL55 4EL.

The sample containing insulin is incubated simultaneously with the three antibodies in the Test Unit. This Test Unit is incubated at 37°C with intermittent agitation, the formation of a soluble sandwich complex occurs only in the presence of Insulin molecules, which bridge the two antibodies. Therefore, only peptides that bridge these

two antibodies can be quantitated. Following this incubation, the Test Unit is spun at high speed about its vertical axis. Reaction fluid is forced up and completely captured in the sump chamber. A series of washes removes the unbound material from the bead and the inner tube. Chemiluminescent substrate is added to the Test Unit. Light emission is read with a high sensitivity photon counter. The amount of alkaline phosphatase captured is directly proportional to the concentration of the analyte in the patient sample.
Sample volume required is 100 µL (250 µL accounting for dead vol.). The minimum detectable concentration is 2 mIU/L.

Intra-assay precision:

	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6
N						
Mean (mIU/L)	7.39	12.3	17.8	25.5	102	300
CV (%)	6.4	5.3	6.1	5.7	5.2	5.3

Total precision - Samples assayed in duplicate over the course of 20 days, two runs per day, for a total of 40 runs and 80 replicates.

	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6
N						
Mean (mIU/L)	7.39	12.3	17.8	25.5	102	300
CV (%)	8.0	6.0	7.1	5.9	6.1	7.0

❖ ***Cortisol***

The assay used to measure cortisol is a competitive immunoassay using chemiluminescent technology on the Advia Centaur (Siemens Medical Diagnostic Solutions, Newbury, UK). Cortisol reagent was supplied by Bayer Diagnostics Europe Ltd.

The Bayer Advia Centaur assay is a competitive immunoassay using direct chemiluminometric technology. Cortisol in the sample is incubated with cortisol labelled with acridinium ester and an anti-cortisol antibody which is covalently bound to parametric particles (PMP). Labelled cortisol competes with cortisol in the patient sample for the limited binding sites on the PMP-bound antibody. After the incubation a magnetic field is applied causing the solid phase (containing the bound labelled cortisol) to be held at the site of the reaction cuvette while the liquid phase is aspirated. The cuvette

is then washed with deionised water. Acid reagent (containing hydrogen peroxide) is then added to the cuvette to begin the light emission reaction with the acridinium ester. The cuvette is then moved to the luminometer and base reagent is added to enhance the light reaction. Light intensity is measured immediately and converted to relative light units. This has an inverse relationship with the cortisol concentration. Sample volume required is 20 µL.

Intra-assay precision

	Level 1	Level 2	Level 3	Level 4	Level 5
Mean (µg/L)	107.05	155.33	390.95	759.55	1024.97
CV%	3.69	3.09	2.89	3.82	2.98

Inter-assay precision

	Level 1	Level 2	Level 3	Level 4	Level 5
Mean (µg/L)	107.05	155.33	390.95	759.55	1024.97
CV%	6.58	4.92	4.22	4.25	4.98

Sensitivity

The minimum detectable concentration is 30 nmol/L.

Assay Range

Serum concentrations up to 1800 nmol/L.

Limitations

Circulating cortisol results from patients receiving Prednisolone or Prednisone (which is converted to Prednisolone *In vivo*) therapy may be falsely elevated. Exercise caution with cortisol determinations for patients undergoing therapy with these and structurally related synthetic corticosteroids.

❖ ***Serum Transferrin Receptor, Ferritin, Full Blood Count***

The Advia 2120 Haematology system was used for the analysis of these parameters (http://diagnostics.siemens.com).

IV Intervention study in school children

IV.1 Letter to the Headteacher

16 September, 2008

KINGS College LONDON
University of London

SCHOOL FOOD TRUST
Eat Better Be Better

Mr David Boyle
Headteacher
Dunraven School
94/98 Leigham Court Road
Streatham, London SW16 2QB

Dear Mr Boyle,

Breakfast eating and learning ability in adolescents

Last summer we carried out research to investigate relationships between the type of breakfast that children eat and how well they do at school. We found that having a larger breakfast that provides a steady release of glucose to the brain selectively enhances performance in the majority of the cognitive function tests that we used (please see abstract attached, which will be published by the Nutrition Society).

I am writing to ask if you would be willing for pupils in your school to take part in a new study, similar to the one we carried out last year. This time, however, rather than have the children tell us what they had for breakfast, we would feed them breakfast ourselves. We could then assess how the type of breakfast affects cognitive function. This is a more powerful experimental design than the previous study. The outcome, of course, is that we may be able to make recommendations to parents and pupils about the types of breakfast associated with better cognitive function. It would also give direction to schools providing breakfast for their pupils.

Please find enclosed a brief summary of the research; a letter, questionnaire, consent form and information sheet for parents; and a consent form and Information Sheet for participants. This research has been approved by the King's College Research Ethics Committee (04/05-105).

I appreciate that teachers face a heavy workload. We have designed the study to avoid any additional workload on teachers. For those pupils willing to take part, we would need about two hours of their time (between 8 and 10 am) on two occasions roughly two weeks apart. The work would be carried out by Renata Micha, a PhD student working under my supervision, together with two research assistants.

I am sure that you believe that we must do all that we can to help children develop and express their full potential at school. I hope that you feel able to support this request for research at Dunraven. Thank you for taking the time to consider this request.

With best wishes,

Dr Michael Nelson
Reader in Public Health Nutrition, King's College London
Head of Research, School Food Trust

PS. If you are willing for your school to be involved in the study or you require further information, please give us a ring on 02078483919/ 07859081757.

IV.2 Parents/ guardians: letter, study information sheet, screening and consent form

November/ 2006,

Dear Parent/Guardian,

Breakfast eating and learning ability in adolescents.

Dunraven School is helping the Department of Nutrition and Dietetics at King's College London and the School Food Trust with research on how the type of breakfast eaten by adolescents may affect how well they do at school. Last summer we carried out research at your school to investigate relationships between the type of breakfast that children eat and how well they do at school. We found that having a larger breakfast that provides a steady release of glucose to the brain selectively enhances performance in the majority of the cognitive function tests that we used.

We are writing to ask if you would be willing for your child to take part in a new study, similar to the one we carried out last year. This time, however, rather than have the children tell us what they had for breakfast, we would feed them breakfast ourselves. We could then assess how the type of breakfast affects cognitive function. This is a more powerful experimental design than the previous study. The outcome, of course, is that we may be able to make recommendations to you and your children about the types of breakfast associated with better cognitive function. It would also give direction to schools providing breakfast for their pupils.

It is very important that we learn more about how the type of breakfast that adolescents eat may affect their learning ability. Each child who takes part in the study will be provided with two different types of breakfast (on two occasions), and will be asked to complete a short questionnaire, tests of learning ability and a mood scale. We also need to take a finger prick blood sample to see how the levels of iron and glucose in the blood affect performance on the tests of learning ability. We will be able to tell you if your child's iron levels are too low. We will write individually to the parents of all students whose blood tests suggest that they have poor iron status. Furthermore, we need to take a saliva sample to see how the stress levels of your child affect her/ his performance.

Please find enclosed an information sheet which explains the research in more detail. We also enclose a separate information sheet for your child.

When you have read the information sheet, we would be grateful if you would complete the enclosed consent form and screening questionnaire and return it to the school in the envelope provided. Co-operation is of course voluntary, and we will be grateful for any assistance that you and your child can give us.

Yours sincerely

Mr David Boyle
Headteacher of Dunraven School

Dr. Michael Nelson
Reader in Public Health Nutrition, KCL
Head of Research, School Food Trust

This study has been approved by the King's College Research Ethics committee: 04/05-105
www.kcl.ac.uk www.schoolfoodtrust.org.uk

INFORMATION SHEET FOR PARENTS

BREAKFAST EATING AND LEARNING ABILITY IN ADOLESCENTS

We would like to invite your child to participate in this research project looking at breakfast consumption and learning ability. Before you decide whether you want your child to take part, it is important for you to understand why the research is being done and what your child's participation will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. If you do decide for your child to take part, please let us know beforehand if he/she has been involved in any other study during the last year.

Why are we carrying out the research?
Recent research in school-aged children shows that breakfast improves school attendance and academic performance. However, the results are not consistent. We found that positive effects of breakfast consumption on learning ability may depend on the type of breakfast rather than the consumption of breakfast itself. We want to know if particular types of breakfast are related to better performance in tests of learning ability. We also want to know if mood is affected by eating breakfast and if it has an effect on the tests of learning ability.

Who will take part in the study?
All the children aged 11-14 in the Dunraven School will be asked to take part. Not all children who consent will be participating, but some who will be selected at random. Children who have any of the following illnesses will not be selected: anaemia, thalassaemia, sickle cell anaemia, other causes of anaemia (hookworm infection, schistostomiasis and malaria), diabetes or other disorders of glucose metabolism, or other acute or chronic illnesses or diseases. The school was selected because the children who attend come from a wide variety of backgrounds. Children can take part when they and their parents or guardians have signed a consent form saying that they understand what the study involves. Any child who starts the study but then changes his/her mind is free to stop at any time without having to give a reason.

What will each child be asked to do?
All children who agree to take part in the study will be asked to be seen three times; on one non-testing occasion and on two testing occasions. All appointments will take place on the same day of the week, but one or two weeks apart. On the non-testing day, children will be seen first thing in the morning for only 15 minutes. We will take a saliva (spit) sample, in order to measure cortisol. Cortisol is used as a marker of stress levels. We want to measure your child's stress levels on a 'normal' day, in order to be able to compare it to the stress levels on a testing day. Your child will also be measured for height and weight, and will be interviewed individually by one of the researchers: Ms Renata Micha, Ms Clare Harper, Ms Yasmin Hosny. This interview will include questions about your child's eating habits (how often he/she eats breakfast, if he/she is a vegetarian etc), his/her physical activity, and his/her current health status. On the non testing morning, children will be asked not to have caffeine and to avoid milk for 30 minutes before sampling (instructions will be given to you). On both testing occasions (two weeks apart), children will be asked to refrain from vigorous activity on the day before the study, to consume the same meal and at the same time on the night before the study (instructions on which foods and drinks to avoid will be given to you), to avoid eating anything else after their last preset meal (with the exception of water), and to sleep a minimum of eight hours. On the day of the study, all eligible children will be provided with breakfast on arrival at school, which will differ on each occasion, will be measured for height and weight, and will be asked some questions about their dinner their night before, the time they slept and their physical activity on that day. Each child will be asked to complete tests of learning ability (for example, tests of memory and concentration) and questions about mood (for example if the child is tired, hungry, happy etc).

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INFORMATION SHEET FOR PARENTS

We will take three finger prick blood samples, one before breakfast, one before and one immediately after completing the tests and mood scales. We will measure haemoglobin and blood glucose levels. We want to measure these because iron status and blood glucose levels are associated with learning ability. There is no risk of infection when blood samples are taken, as there will only be used sterilized equipment and disposable lancets. We will also take three salivary measures of cortisol; once before breakfast, and then before and after the tests. We want to measure cortisol, because stress levels may also affect the outcome of the tests. Each testing session will take approximately two and a half hours, and it will take place during lesson time. We will write to you if your child's blood tests suggest that they have poor iron status. A snack will be provided after each testing day (e.g. apple, banana).

Who will see this information?
Only the people from King's College London and the School Food Trust who are directly involved in the research will see the information for individual children. The information collected will not be shown to anyone else. All the information collected will be kept strictly confidential. No individual child will be identifiable in any verbal or written reports about the research. At the end of the study, each child and his/her parents or guardians will be told about the results of the learning ability tests and whether the type of breakfast affects the students' performance on these tests. Parents will also be informed if their child has low iron levels.

When will the work be carried out?
The study will be carried out between December 2006 and March 2007 by researchers at King's College London. If you have any questions about the study, please ring 02078483919/ 02078484349.

It is up to you to decide whether or not your child should take part. If you do decide for your child to take part you will be given this information sheet to keep and be asked to sign a consent form and fill in a screening questionnaire; the questionnaire is likely to take you 20 minutes to fill in. You may freely withdraw your child at any time and without giving a reason, even when parental consent has already been given. In the event of your child suffering any adverse effects as a consequence of his/her participation in this study, you will be compensated through King's College London's 'No Fault Compensation Scheme'.

For further information please contact:
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Department of Nutrition and Dietetics
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London SE1 9NH
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e-mail: michael.nelson@kcl.ac.uk

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PARENT OR GUARDIAN SCREENING AND CONSENT FORM

BREAKFAST EATING AND LEARNING ABILITY IN ADOLESCENTS

I have read and understood the study information sheet overleaf.

I understand that I may freely withdraw my child from the study at any time without giving a reason, even when parental consent has already been given. I understand that all information collected for the purposes of this research study will be treated as strictly confidential and handled in accordance with the 'Data Protection Act 1998'.

I do/do not* consent for my child (Name): _____

Gender of child: Male/ Female

Form: _____ to take part in this study.
* please delete as appropriate

Name of parent or guardian: _____

Signature of parent or guardian: _____

Date: _____

Phone number: _____ (day)
_____ (evening)
_____ (mobile)

If you are willing for your child to participate in this study, please complete the following questions on the following pages.

When you have completed the form, please return it to the school in the envelope provided as soon as possible.

PARENT OR GUARDIAN SCREENING AND CONSENT FORM

1. Does your child have or is there a family history of any of the following conditions?
(the conditions listed here are the exclusion criteria for the study)
Please tick (√) one box or two boxes in every row

	Yes, child has condition	Yes, family history of condition	Neither
Sickle-cell anaemia			
Haemophilia			
Thalassaemia traits			
Any other blood disorder (please specify)			
Colour blindness			
Diabetes			
Any other glucose toleran (please specify)			
Any other chronic disease (please specify)			

2. Does your child take any medication prescribed by a doctor on a regular basis?
Please tick (√) ONE box

Yes ☐

No ☐

If YES:
Please give details:

Type	Dosage	Reason for taking it	How often & time of the day	How many tablets

3. Does your child have any learning disabilities/ mood disorders?
Please tick (√) ONE box

Yes ☐

No ☐

If YES, please state them:

_____

_____

PARENT OR GUARDIAN SCREENING AND CONSENT FORM

4. Is your child on a special diet for medical reasons?

Please tick (√) ONE box

Yes ☐

No ☐

If YES, please give details:

Description of diet	Why is your child on this diet?

5. In the past four weeks, has your child had any infections?

Please tick (√) ONE box

Yes ☐

No ☐

If YES, please give details:

Type of infection	When was that? (date of appearance)	Why	How long did it last?

6. Is your child taking any vitamin or mineral tablets/ supplements or herbal products now?

Please tick (√) ONE box

Yes ☐

No ☐

If YES: Please give details:

Type	Dosage	Reason for taking it	How often & time of the day	How many tablets

PARENT OR GUARDIAN SCREENING AND CONSENT FORM

7. **Does your child follow a special diet for religious reasons?**

Please tick (√) ONE box

Yes ☐

No ☐

If YES, please give details:

_____

_____

_____

8. **Does your child have any food intolerances/ allergies?**

Please tick (√) ONE box

Yes ☐

No ☐

If YES, please give details:

_____

_____

_____

9. **Is your child a vegetarian?**

Please tick (√) ONE box

Yes ☐

No ☐

If YES, please give details (name the types of food your child does not eat):

_____

_____

_____

10. **How often does your child have breakfast?**

Please tick (√) ONE box

Never ☐

Once a week ☐

Twice or more a week ☐

Every day ☐

Other, please state _____ ☐

If your answer is NEVER, please go directly to question number 12.

PARENT OR GUARDIAN SCREENING AND CONSENT FORM

11. **If your child has breakfast (even if not every day), please answer the following questions:**

a. What does your child typically have for breakfast?

FOOD/BEVERAGE	AMOUNT

b. Please write down the time that your child typically has breakfast:

time your child starts eating breakfast: _____ (AM)

time your child finishes eating breakfast: _____ (AM)

12. **Will you be willing for your child to have the following breakfast meals?**

Breakfast meal 1	Breakfast meal 2
Muesli	Corn flakes
Semi-skimmed milk	Semi-skimmed milk
Apple juice	Apple juice

Please tick (√) ONE box

Yes ☐

No ☐

13. **a. What does your child typically have for dinner (evening meal)?**

FOOD/BEVERAGE	AMOUNT A

b. Please write down the time that your child typically has dinner (evening meal):

time you start eating dinner: _____ (PM)

time you finish eating dinner: _____ (PM)

PARENT OR GUARDIAN SCREENING AND CONSENT FORM

14. What time does your child typically go to sleep every night, and what time does your child typically wake up in the mornings?

a. time your child sleeps _____ (PM)

b. time your child wakes up _____ (AM)

15. Please write down your child's current weight.

stones: _____ pounds: _____ OR kilos: _____

16. Please write down your child's birth weight.

pounds: _____, OR kilos: _____

17. Please write down your child's height.

feet: _____ inches: _____ OR cm: _____

18. Please write down your child's date of birth.

Date of birth: _____ (Day/ Month/ Year)

19. Does your child exercise?

Please tick (√) ONE box

Never	☐
Once a week	☐
Twice or more a week	☐
Every day	☐
Other, please state _____	☐

If your child DOES exercise, please give details for each one of your child's activities:

Type of exercise	How long per time	Intensity (low, moderate, high, or very high)	How often per week (once a week, twice or more a week, every day, other state)

PARENT OR GUARDIAN SCREENING AND CONSENT FORM

20. Which of the following do you think best describes your child's ethnic origin?

Please tick (√) ONE box

White Caucasian	☐	Pakistani	☐
Black-Caribbean	☐	Bangladeshi	☐
Black-African	☐	Chinese	☐
Black-Other	☐	Asian-Other	☐
Indian	☐	Other, please specify	☐

21. Is English your child's first language?

Please tick (√) ONE box

Yes ☐

No ☐

If NO:
Please write down your child's first language: _____

22. Does your child smoke?

Please tick (√) ONE box

Yes ☐

No ☐

Don't know ☐

If YES, please give details:

How often	Cigarettes/ day

PARENT OR GUARDIAN SCREENING AND CONSENT FORM

23. If your child is a girl, please answer the following questions. Otherwise, please go directly to question number 24.

Has your daughter started her periods?
Please tick (√) ONE box

Yes ☐

No ☐

If YES,

a) How old was your daughter when they started?

_____ years _____ months

b) How often does your daughter have her periods?
Please tick (√) ONE box

Regularly, about once a month ☐

Irregularly ☐

c) When was the start of your daughter's most recent period?
Please tick (√) ONE box

This week ☐

About 1 week ago ☐

About 2 weeks ago ☐

About 3 weeks ago ☐

About 4 weeks ago ☐

24. Is your daughter taking contraceptive pills?
Please tick (√) ONE box

Yes ☐

No ☐

Don't know ☐

PARENT OR GUARDIAN SCREENING AND CONSENT FORM

25. Please answer the following questions regarding education and occupation for yourself and (if applicable) your partner.

a. Are you working?

Father		Mother	
Yes	☐	Yes	☐
No	☐	No	☐

If NO, does any of the following apply to you?

b. Are you in full-time education?

Father		Mother	
Yes	☐	Yes	☐
No	☐	No	☐

c. What was the highest level of education that you reached?

	Father	Mother
Higher degree (e.g., Masters, PhD)	☐	☐
Degree/HND	☐	☐
A Level/ BTEC/City & Guilds/ HNC	☐	☐
GCSE/O Levels/ GNVQ	☐	☐
Other, please state _____	☐	☐

PARENT OR GUARDIAN SCREENING AND CONSENT FORM

d. Please write down the age and occupation of yourself and (if applicable) your partner, noting whether you work as an employee or are self-employed, and if your work involves supervising others.
If you are not working at the moment, please provide this information for your last main job.

	Father	Mother
Age (years)		
Occupation:		
Occupation/ job title		
Type of industry		
Occupation description/ main role		
Employee/self-employed		
Manage other employees (Yes/ No); If yes, please state the number of people you manage		
Supervisory (Yes/No) If yes, please state the number of people you supervise		

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE

ALL INFORMATION IS CONFIDENTIAL, FOR RESEARCH PURPOSES ONLY

Please enclose this form in the envelope provided and return it to the school as soon as possible.

IV.3 Participants: study information sheet, consent form

INFORMATION SHEET FOR PARTICIPANTS

BREAKFAST EATING AND LEARNING ABILITY IN ADOLESCENTS

We would like to invite you to take part in some research looking at breakfast eating and how well you learn. You should only take part if you want to. If you choose not to take part, you will not be affected in any way. Before you decide whether you want to take part, it is important for you to understand why the research is being done and what you will be asked to do. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. If you do decide to take part, please let us know beforehand if you have been involved in any other study during the last year.

Why are we carrying out the research?
Recent research shows that school-aged children who eat breakfast have better school attendance and better grades. However, the studies do not all agree on the effects of eating breakfast. We found that positive effects of breakfast eating on learning ability may depend on the type of breakfast rather than simply whether or not breakfast was eaten. We want to know if particular types of breakfast are related to better performance in tests of learning ability. We also want to know if mood is effected by eating breakfast and if it has an effect on the tests of learning ability.

Who will take part in the study?
All children aged 11-14 in your school will be asked to take part. The school was selected because the children who attend come from a wide variety of backgrounds. You will be allowed to take part when you and your parents or guardians have signed consent forms saying that you understand what the study involves. You will not be allowed to take part in the study if you have anaemia or diabetes. If you start the study but then change your mind, you are free to stop at any time without having to give a reason. A decision to stop will not effect you in any way.

What will you be asked to do?
If you agree to take part in the study, you will be asked to be seen on three ocassions. On your first appointment ('normal' day) we will see you first thing when you arrive at school for 15 minutes, and we will ask you to give us a saliva (spit) sample. This will allow us to measure your stress levels on that morning. On that morning you can have your breakfast, but you shouldn't have anything else 30 minutes before we see you. Instructions will be given to you. You will be measured for height and weight and you will be interviewed individually by either Ms Renata Micha, Ms Clare Harper, or Ms Yasmin Hosny about what you usually eat (for example, how often you eat breakfast, if you are a vegetarian), your physical activity, and your current health. These questions will be similar to the ones that we are going to ask your parents or guardians to complete.

This study has been approved by the King's College Research Ethics Committee, reference 04/05-105

INFORMATION SHEET FOR PARTICIPANTS

On the other two occasions ('breakfast' days) you will be given breakfast. You will be measured for height and weight and asked some questions about your dinner the night before, the time you slept, and your physical activity on that day. On the evening before your 'breakfast' day you will be given a short list of foods and drinks that you should avoid eating and drinking on that evening. In addition, on the day before your 'breakfast' day you will be asked to avoid strenuous exercise, and to have a good night's sleep. On each of your 'breakfast' days you will be asked to complete tests of learning ability (for example, tests of memory and concentration) and questions about mood (for example, if you are tired, hungry, happy or sad). We will take three finger prick blood samples, one before breakfast, one before and one immediately after completing the tests and the questions about mood. We will measure the amount of iron in your blood, and your blood sugar levels. We want to measure these because iron and sugar levels in blood (as well as breakfast) may affect mood and the outcome of the tests. There is no risk of infection when the blood samples are taken, since all the equipment we use is sterilized. We will also measure your stress levels by taking a saliva sample; once before breakfast, and then before and after the tests. Stress levels may also affect the outcome of the tests. At the end of each appointment a snack will be provided (e.g. banana).

Who will see this information?
Only the people from King's College London and the School Food Trust who are directly involved in the research will see the information about you. The information collected will not be shown to anyone else. All the information provided by you will be kept strictly confidential, and will not be available to your parents. You will not be identifiable in any verbal or written reports about the research. At the end of the study, you and your parents or guardians will be told about the results of your own tests and whether in general the type of breakfast that is eaten affects performance levels.

When will the work be carried out?
The study will be carried out between December 2006 and March 2007 by Ms Renata Micha, Ms Clare Harper, and Ms Yasmin Hosny, researchers at King's College London. If you have any questions about the study, please ring 020 7848 3919.

Thank you.

This study has been approved by the King's College Research Ethics Committee, reference 04/05-105

IV.4 Appointment form: screening day

BREAKFAST & LEARNING STUDY

INSTRUCTIONS SHEET - 'normal' day

Name: _____

Form: _____

Your 'normal' day appointment is on: _____

Please, meet us in front of Ms Lowes' office at 8:10 am *(reception area)*.

Please, read the instructions carefully.

<u>On the day before each appointment, please:</u>

- Eat your normal diet, and follow your normal routine.
- Have a good night's sleep (~ 8 hours).

<u>On the morning of your appointment:</u>

- You can have your normal breakfast if you wish.
- Please, don't eat or drink any caffeinated products for 2 hours before your appointment (eg coffee, tea, cola drinks, chocolate).
- Please, don't drink or eat anything 30 minutes before your appointment.
- Avoid any form of strenuous physical activity; please come to school using the least strenuous means of transport.
- Come to school at 8:10 am.
- You will meet either Renata, Clare or Yasmin at reception.

For you to complete:

On the day of your appointment (as shown above), if you have breakfast, please fill in the table below and bring this form with you to your appointment.

BREAKFAST

Time started eating breakfast	AM
Time finished eating breakfast	AM

We look forward to meeting you.

Kind regards,

Renata, Clare, Yasmin.

If you have any questions, please feel free to contact us at any time:

Renata Micha

E-mail: eirini.micha@kcl.ac.uk

Tel: 020 7848 3919/ 07859001757

IV.5 Appointment form: testing day

Name: _____

Form: _____

Your 'breakfast' day Appointments are on:

(1st) _____ AND (2nd) _____

Please, meet us in front of Ms Lowes' office at 8:10 am

(reception area).

DO NOT eat or drink anything on both of these mornings after waking up (you can only have water)! If you do eat or drink anything, you WILL NOT be able to take part on that day.

Please, read the instructions on both pages carefully.

On the day before each appointment, please:

- Eat your normal diet.
- Avoid alcohol consumption.
- Restrict the consumption of caffeine containing products (eg coffee, tea, cola drinks, chocolate).
- Restrict your participation in intense physical activity (eg long periods at the gym, excessive swimming, running, aerobics)
- Have a dinner/ evening meal of your choice, but this must be the same meal before each appointment, and has to be eaten at the same time. Please write down what you ate and the quantity and bring this information with you *(see page 3)*. Your dinner/ evening meal should be finished by 9:00 pm the latest.
- It is important that you have dinner/ evening meal the evening before your appointment. An example of dinner is: meat (eg chicken, turkey, beef) with salad or vegetables (raw or boiled) and rice (or pasta, or potatoes). Avoid fried ready meals.
- Consume dinner in about 20 min, and sit quietly after dinner and before bed.
- Don't eat or drink anything else after 9 pm. You can have water, but in moderation.
- Have a good night's sleep (~ 8 hours).

<u>On the morning of the study, please:</u>

- Complete page 3 of this sheet and bring it with you to your appointment.
- Don't eat or drink anything. You can have water in moderation, as long as it is 30 - 45 min before your appointment.
- Avoid any form of strenuous physical activity; please come to school using the least strenuous means of transport.
- Come to the school at 8:00 am. You will meet either Renata, Clare, Ellen or Caroline at reception.
- You can bring something to read/ homework to complete, as there might be some free time during the testing period.

<u>For you to complete:</u>

The evening before your appointment please fill in the table below, and bring this form with you to your appointment.

<u>DINNER/ EVENING MEAL</u>

Time started to eat dinner	PM
Time finished eating dinner	PM

Please, write down what you had.

FOOD/BEVERAGE	AMOUNT

We look forward to seeing you again.

Kind regards,

Renata, Clare, Yasmin.

IV.6 Researcher's booklet: non-testing day

Today's date: _____ (Day/ Month/ Year)
Time on arrival: _____ AM
Form class: _____
Date of birth: _____ (Day/ Month/ Year)
Gender: Male/ Female (please circle)

<u>EXCLUSION CRITERIA</u>

1. **Did you follow the instructions that were given to you?** ***(including the instructions about the saliva sample)***

Yes ☐

No ☐

If, No please explain _____

2. **Did you have anything to eat/ drink since you woke up this morning?**

(Probe: breakfast; ask the child to give you his/ her instructions sheet, and fill it in accordingly)

Yes ☐

No ☐

Time started to eat breakfast: _____ AM
Time finished eating breakfast: _____ AM

3. **What was the last thing you had before you came here?**

(Probe: It doesn't have to be a meal, it could be sth that a friend gave to them, on their way to school, at school, a sweet, a candy, drink – half a can of coke, bread, milk, juice, fruit etc)

Yes ☐

No ☐

Record amount after sample collection

_____

_____

What time did you have it? _____ (AM)

4. **Did you have tea, coffee, coke, chocolate, red bull or an alcoholic drink this morning?**
(probe: added sugar, milk etc/ at home – on their way to school – at school)

Yes ☐

No ☐

If YES, please tell me how much and what time:

Description of drink	Time (AM)	Amount in household measures or photo

The child should not have had caffeinated products for 2 hours before sampling.

A child who has eaten < 30 minutes prior to sampling may be asked to rinse off his or her mouth and to wait for 3 to 5 minutes

(long enough to re-establish the natural oral environment and a salivary pH of about 6.4 – 7.4).

5. **Did the child have to rinse his/ her mouth?**

Yes ☐

No ☐

Record time: _____ (AM)

SALIVA SAMPLE

Take the saliva sample (the child should keep the swab in their mouth until they feel they can no longer prevent themselves from swallowing the saliva produced; approximately 1 to 2 min)

	RECORD TIME (AM)	RECORD TIME (AM)	VALUE
CORTISOL	*when in mouth*	*when out of mouth*	*(following biochemical analysis)*

SCREENING ON THE DAY

Check whether the child is feeling alright (to see whether they are not feeling well. That will affect their cortisol levels.

1. **Are you feeling healthy and well today?**
Yes
No (explain) _____

2. **Was today a typical day at home?**
Yes
No (explain) _____

3. **Did you have an emotional event today before coming here (such as fighting with sibling – parents, prolonged crying for more than 10 minutes)?**
Yes/ No **(please circle)**
If Yes, (explain) _____ **(what kind of event)**
_____
At what time? _____ am
How long did it last? _____ min

4. **Did you participate in any vigorous physical activity today before coming here?**

Yes ☐

No ☐

What type of exercise?	When did it start? (AM)	For how long?	Intensity (low, moderate, high, very high)

(record walking if >10 min, and running if > 5 min)

5. Have you had anything to drink and/or eat since you woke up this morning? Check exclusion criteria question no 1 and ask accordingly.

(Probe: It doesn't have to be a meal, it could be sth that a friend gave to them, on their way to school, at school, a sweet, a candy, drink – half a can of coke, bread, milk, juice, fruit etc; added sugar, milk semi-skimmed etc)

Yes ☐

No ☐ .

If YES, please tell me what you had and roughly what time.

FOOD/ BEVERAGE	AMOUNT (food atlas)	TIME (AM)

What time was it when you finished eating?: _____

6. Can you think of anything else that you had to drink/eat on your way to school?

(Probe: This might include anything that you bought or that a friend gave you)

Yes ☐

No ☐

If YES, please tell me what you had and roughly what time.

Description of food or drink	Time	Amount in household measures or photo

What time was it when you finished eating?: _____

7. Can you think of anything else that you had to drink/eat since you came to school?

(Probe: This might include anything that you bought or that a friend gave you)

Yes ☐

No ☐

If YES, please tell me what you had and roughly what time.

Description of food or drink (at school)	Time	Amount in household measures or photo

What time was it when you finished eating? _____

8. How often do you have breakfast?

Please tick (√) ONE box

Every day ☐

Twice or more a week ☐

Once a week ☐

Never ☐

Other, please state _____ ☐

9. a. Are you a vegetarian?

Please tick (√) ONE box

Yes ☐

No ☐

If NO, please go to question 10, page 7.

If YES:

b. **Do you sometimes eat fish?**

Please tick (√) ONE box

Yes ☐

No ☐

d. **How long have you been a vegetarian?**

Please tick (√) ONE box

Less than a year ☐

1 – 5 years ☐

More than 5 years ☐

Always ☐

d. **Why are you a vegetarian? (if more than 2 reasons, number the boxes in order of importance from 1 to 5)**

My family is vegetarian ☐

I do not like meat ☐

I do not agree with killing animals ☐

Meat is fattening ☐

Health reasons ☐

Other, please state _____ ☐

10. **On average, how often do you have the following foods or drinks?**

Please tick (√) ONE box in every row

	Never	Once a week	Twice or more a week	Once a month
Red Meat				
Other types of meat or meat products (eg sausage rolls, steak and kidney pie)				
Chicken & turkey				
Eggs (e.g. in omelettes or sandwiches)				
Burgers (meat)				
Spaghetti Bolognese & lasagne (meat)				
Beans & lentils				
Fish or fish products (e.g. fish fingers)				
Tea				
Coffee				
Coke, Pepsi, or other cola drinks				
Red Bull				
Alcohol (beer, cider, wine, alcopops, etc.)				

11. **Do you follow a special diet for religious reasons?**

Please tick (√) ONE box

Yes ☐

No ☐

If YES, please give details:

_____

_____

_____

_____

12. **Are you taking vitamin or mineral tablets/ supplements now?**

Please tick (√) ONE box

Yes ☐

No ☐

If YES, please tell me what type of supplements you take and how often you take them:

Type *(if they don't remember ask them to find out)*	Dosage	Reason for taking it	How often (every day, twice or more a week, once a week, never, other please state)	Time of the day	How many tablets

13. Are you on a special diet for medical reasons?

Please tick (√) ONE box

Yes ☐

No ☐

If YES, please describe this diet and say why you are on it:

_____

_____

_____

_____

14. Do you take any medication prescribed by a doctor on a regular basis, or have you just started taking any?

Please tick (√) ONE box

Yes ☐

No ☐

If YES, please tell me what type and why:

Type *(if they don't remember ask them to find out)*	Dosage	Reason for taking it	How often (every day, twice or more a week, once a week, never, other please state)	Time of the day	How many tablets

15. In the past four weeks, have you had any infections?

Please tick (√) ONE box

Yes ☐

No ☐

If YES, please give details:

Type of infection	When was that? (date of appearance)	Why	How long did it last?

16. Have you gone on a diet to LOSE weight in the past year?

Please tick (√) ONE box

Yes ☐

No ☐

17. Have you deliberately tried to GAIN weight in the past year?

Please tick (√) ONE box

Yes ☐

No ☐

18. a. Has your weight changed in the past year by more than a stone (14 pounds or six kilos)?

Please tick (√) ONE box

Yes ☐

No ☐

Don't know ☐

If YES:

b. Did you lose or gain weight?

Please tick (√) ONE box

Lose ☐

Gain ☐

If YES, please give details *(probe: how long, by a dietician/ personal diet, types of food avoided or excluded, weight lost/ gained in the last 6 months)*:

_____

_____

_____

_____

19. **How would you describe your body weight?**

Please tick (√) ONE box

Very underweight ☐

Slightly underweight ☐

About right ☐

Slightly overweight ☐

Very overweight ☐

20. **What time did you go to sleep last night, and what time did you wake up this morning?**

a. Time you slept _____ (pm)

b. Time you woke up _____ (am)

21. **Do you exercise?**

Please tick (√) ONE box

Yes ☐

No ☐

If you DO exercise, please give details:

What type of exercise?	When did it start? (AM/ PM)	For how long?	Intensity (low, moderate, high, very high)	How often (once a week, twice or more a week, every day, other state)

(record walking if >10 min, and running if > 5 min)

22. **Do you have TV set in your bedroom?**

Please tick (√) ONE box

Yes ☐

No ☐

TIME NOW: _____ **AM**

FOR GIRLS ONLY

23. a. Have you started your periods?

Please tick (√) ONE box

Yes ☐

No ☐

(If NO, please go directly to question 26)

c. If YES, how old were you when they started?

_____ years _____ months OR _____ (date, MM/YYYY)

d. How often do you have your periods?

Please tick (√) ONE box

Regularly, about once a month ☐

Irregularly ☐

24. a. Are you having your period currently?

Please tick (√) ONE box

Yes ☐

No ☐

b. If YES, when did it start? _____ (date, DD/MM/YYYY)

c. If NO, are you expecting your period within the next 5 days?

Please tick (√) ONE box

Yes ☐

No ☐

25. When was the start of your most recent period?

Please tick (√) ONE box

This week ☐

About 1 week ago ☐

About 2 weeks ago ☐

About 3 weeks ago ☐

About 4 weeks ago ☐

26. Are you taking contraceptive pills?

Please tick (√) ONE box

Yes ☐

No ☐

If, YES when did you start taking them? _____ (MM/YYYY)

(date you started taking them)

_____ (type of)

TIME NOW: _____ AM

HEIGHT AND WEIGHT MEASUREMENTS

HEIGHT: 1)_____, 2)_____ 3)_____CM

Take only two measurements if they differ ≤ 0.5 cm; otherwise take a third measure

WEIGHT: 1)_____, 2)_____ 3)_____KG

Take only two measurements if they differ ≤ 0.1kg; otherwise take a third measure

Scales: A/ B (please circle)

TIME NOW: _____ AM

Make the other two appointments with the child and give her/ him the instructions sheet.

IV.7 Researcher's booklet: testing day

Today's date: _____ (Day/ Month/ Year)

Time on arrival: _____ AM

Form class: _____

Date of birth: _____ (Day/ Month/ Year)

Gender: Male/ Female (please circle)

No for sequencing: _____ (from 1 to 64) **Pair:** _____ (from 1 to 32)

Visit 1st 2nd (please circle)

GL: Low High (please circle)

GI: Low High (please circle)

Breakfast meal: 1 2 3 4 (please circle)

Version of the CF tests: 1 2 (please circle)

ON ARRIVAL

EXCLUSION CRITERIA

5. **Did you follow the instructions that were given to you?** *(including the instructions about the dinner; ask them to give you their recorded dinner)*

Yes

No (explain) _____

6. **Have you had anything to drink and/or eat since you woke up this morning? At home? On your way to school? Since you came to school?**

(Probe It doesn't have to be a meal, it could be sth that a friend gave to them, on their way to school, at school, a sweet, a candy, drink – half a can of coke, bread, milk, juice, fruit etc added sugar, milk semi-skimmed etc)

Yes/ No

a. Did you have any water? *(record amount of water as well)* Yes/No

b. If YES, please tell me what you had and roughly what time; exclude them accordingly.

FOOD/ BEVERAGE	AMOUNT (food atlas)	TIME (AM)

What time was it when you finished eating?: _____

c. If YES, was that the last thing you had before you came here?

Yes ☐

No ☐

FOOD/ BEVERAGE	AMOUNT (food atlas)	TIME (AM)

What time was it when you finished eating? _____

7. **Did you have tea, coffee, coke, red bull or an alcoholic drink this morning?**

Please tick (√) ONE box

Yes ☐

No ☐

IF YES, please tell me what you had and roughly what time; exclude them accordingly.

(probe: added sugar, milk etc/ at home – on their way to school – at school)

Description of drink	Time	Amount in household measures or photo

SALIVA SAMPLE

Take the first saliva sample (the child should keep the swab in their mouth until they feel they can no longer prevent themselves from swallowing the saliva produced; approximately 1 to 2 min)

	RECORD TIME (AM)	RECORD TIME (AM)	VALUE
CORTISOL	*when in mouth*	*when out of mouth*	*(following biochemical analysis)*

SCREENING ON THE DAY

Check whether the child is feeling alright (to see whether they are not feeling well). ALSO, check the baseline mood scales – that would give an indication of how they are feeling.

a. **Are you feeling healthy and well today?**

Yes

No (explain) _____

b. **Was today a typical day at home?**

Yes

No (explain) _____

c. **Did you have an emotional event today before coming here (such as fighting with sibling – parents, prolonged crying for more than 10 minutes)?**

Yes/ No (please, circle)

If Yes, (explain) _____ (what kind of event)

_____

At what time? _____ am

How long did it last? _____ min

d. **Did you participate in any physical activity today before coming here?**

Yes/ No (please, circle)

Type of exercise	Time (am) (that the activity started)	For how long?	Intensity (low, moderate, high, very high)

(record walking if >10 min, and running if > 5 min)

BASELINE MEASUREMENTS

	RECORD TIME (AM)	VALUE		
BLOOD GLUCOSE	*finger prick time*	mmol/l	mmol/l	mmol/l
HAEMOGLOBIN	*finger prick time*			g/L or g/dL

Glucose meter: 1 2 3 4 (please circle)

HemoCue: A B (please circle)

BASELINE MOOD SCALES

	START TIME (am)	FINISH TIME (am)	TIME TO COMPLETE *(time with stopwatch)*
Mood scales-baseline			

BREAKFAST – TIME ZERO

You should now set the main timer (90 minutes later starts the testing)

	START EATING (am)	FINISH EATING (am)	TIME TO COMPLETE *(time with stopwatch)*	Comment (if they didn't eat the entire breakfast)
Breakfast				

HEIGHT AND WEIGHT MEASUREMENTS

HEIGHT: 1)_____, 2)_____ 3)_____CM

Take only two measurements if they differ ≤ 0.5 cm; otherwise take a third measure

WEIGHT: 1)_____, 2)_____ 3)_____KG

Take only two measurements if they differ ≤ 0.1kg; otherwise take a third measure

Scales: A/ B (please circle)

TIME NOW: _____ AM

INTERVIEW QUESTIONS

TIME NOW: _____ **AM/ PM**

Please, remember that everything you say to me is strictly confidential, and this information will not be shown to anyone else!

1. What was the last thing you had to drink/eat last night (ask the child to give you his/her sheet where they recorded their dinner)?
(prompt: dinner/ evening meal)

Description of food/ drink	Amount in household measures or photo

What time was it when you started eating? _____ **(pm)**
What time was it when you finished eating? _____ **(pm)**

2. Can you think of anything else that you had to drink/ eat after that? Before you went to bed?
(prompt: fruit, biscuits, tea, milk, etc)

Description of food/ drink	Time	Amount in household measures or photo

What time was it when you finished eating? _____

3. Are you taking vitamin or mineral tablets/ supplements now? Have you started taking any?

Please tick (√) ONE box

Yes ☐

No ☐

If YES: Please tell me what type of supplements you take and how often you take them:

Type *(if they don't remember ask them to find out)*	Dosage	Reason for taking it	How often (every day, twice or more a week, once a week, never, other please state)	Time of the day	How many tablets

4. Are you on a special diet for medical reasons?

Please tick (√) ONE box

Yes ☐

No ☐

If YES, please describe this diet and say why you are on it:

_____

_____

_____

5. Do you take any medication prescribed by a doctor on a regular basis, or have you just started taking any?

Please tick (√) ONE box

Yes ☐

No ☐

If YES:

Please tell me what type and why:

Type *(if they don't remember ask them to find out)*	Dosage	Reason for taking it	How often (every day, twice or more a week, once a week, never, other please state)	Time of the day	How many tablets

6. In the past two weeks, have you had any infections?

Please tick (√) ONE box

Yes ☐

No ☐

If YES, please give details:

Type of infection	When was that? (date of appearance)	Why	How long did it last?

7. Did you have any physical exercise yesterday?

Please tick (√) ONE box

Yes ☐

No ☐

What type of exercise?	What time was that? (AM/ PM) (when it started)	For how long?	Intensity (low, moderate, high, very high)

(record walking if >10 min, and running if > 5 min)

8. What time did you go to sleep last night, and what time did you wake up this morning?

a. time you slept _____ (pm)

b. time you woke up _____ (am)

TIME NOW: _____AM

FOR GIRLS ONLY

Check from the non-testing day whether the girl has started her period. If she reported yes, go straight to question no 10.

9. a. Have you started your periods?

Please tick (√) ONE box

Yes ☐

No ☐

d. If YES, how old were you when they started?

_____ years _____ months OR _____ date

e. How often do you have your periods?

Please tick (√) ONE box

Regularly, about once a month ☐

Irregularly ☐

10. a. Are you having your period currently?

Please tick (√) ONE box

Yes ☐

No ☐

b. If YES, when did it start? _____

c. If NO, are you expecting your period within the next 5 days?

Please tick (√) ONE box

Yes ☐

No ☐

11. **When was the start of your most recent period?**
Please tick (√) ONE box

This week	☐
About 1 week ago	☐
About 2 weeks ago	☐
About 3 weeks ago	☐
About 4 weeks ago	☐

12. **Are you taking contraceptive pills?**
Please tick (√) ONE box

Yes	☐
No	☐

If, YES how long have you been on them? _____ **(MM/YYYY)**

(date you started taking them)

_____ **(type of)**

TIME NOW: _____ **AM**

They are not supposed to have anything else after breakfast, including water. Nonetheless, if they are thirsty and they have water, record the amount.

13. **Did the child have water?** **Yes/** **No** **(please circle)**

Drink	Time (am)	Amount from Atlas

90 MINUTES AFTER THE START OF BREAKFAST

MEASUREMENTS BEFORE THE TESTING

	RECORD TIME (AM)	RECORD TIME (AM)	VALUE		
CORTISOL	*when in mouth*	*when out of mouth*	*(following biochemical analysis)*		
BLOOD GLUCOSE	*finger prick time*		mmol/l	mmol/l	mmol/l
HAEMOGLOBIN	*finger prick time*		g/L or g/dL		

Glucose meter: **1** **2** **3** **4** **(please circle)**

HemoCue: **A** **B** **(please circle)**

MOOD SCALES AND COGNITIVE FUNCTION TESTS

	START TIME hh:mm:sec (from beginning of breakfast)	FINISH TIME hh:mm:sec (from beginning of breakfast)	TIME TO COMPLETE *(time with stopwatch)*
Mood scales - before			
Word generation task			*2 min*
Word recall -immediate			*45 sec* *2 min*
Stroop task			
Easier (control) test			
Harder (actual) test			
Matrices			*6 min*
Speed of information processing			*3 min*
Serial Sevens			*3 min*
Word recall - delayed			*2 min*
Mood scales - after			
Task demand			

* *For all tests & the mood scales, time starts from the moment you say GO, and the student starts the test (this means that you* ***don't time the introductory session****). For the task demand questions, time starts from the beginning,* ***including the introductory sessions*** *for each one of the three questions.*

* *Fixed time*

MEASUREMENTS AFTER THE TESTING

	RECORD TIME (AM)	RECORD TIME (AM)	VALUE		
CORTISOL	*when in mouth*	*when out of mouth*	*(following biochemical analysis)*		
BLOOD GLUCOSE	*finger prick time*		mmol/l	mmol/l	mmol/l
HAEMOGLOBIN	*finger prick time*				g/L or g/dL

Time now: _____ **am**

THE END!!!

IV.8 Certificate of attendance

KING'S College LONDON

University of London

This is to certify that

_____

took part in

The Breakfast and Learning Study

November 2006-June 2008

IV.9 Macronutrient composition of the breakfast meals administered

	BREAKFAST MEALS			
	HIGH GL		LOW GL	
	Low GI (M1)	High GI (M2)	Low GI (M3)	High GI (M4)
GI meal	48	61	48	61
GL meal	41	55	21	28
Energy (kcal)	469.7	468.6	281.2	275.6
Energy (Kjoul)	1965.1	1960.6	1176.5	1153.1
Protein (g)	13.9	14.0	12.5	12.0
Fat (g)	7.1	5.3	6.4	5.1
• of which saturates (g)	2.7	3.4	3.0	3.4
Total CHO (g)	86.6	90.4	43.2	45.2
• of which sugar (g)	54.7	48.6	23.9	22.4
• of which starch (g)	31.9	41.8	19.4	22.8
Sugar breakdown				
• NMES (g)	37.0	30.5	6.7	5.7
• Intrinsic & Milk Sugar (g)	17.7	18.1	17.2	16.7
Sugar breakdown				
• Glucose (g)	9.8	6.4	1.6	0.3
• Fructose (g)	20.3	14.6	1.8	0.3
• Sucrose (g)	10.0	10.8	5.0	5.7
• Maltose (g)	0.0	0.0	0.0	0.0
• Lactose (g)	11.8	15.0	13.6	15.0
• Other Sugars (Oligos) (g)	3.1	2.1	1.9	1.1
NSP (g)	5.1	1.7	3.1	0.9
Total Wt (g)	112.7	111.3	65.2	63.2

IV.10 Biochemical assay for the measurement of salivary cortisol

Salivary Cortisol ELISA kit is supplied by DRG International, Frauenbergstr. 18, D-35039 Marburg-Germany. The DRG salivary cortisol ELISA kit is based on the competition principle and microplate separation. An unknown amount of cortisol present in the sample and a fixed amount of cortisol conjugated with horse-radish peroxidase (HRP) compete for the binding site of mouse monoclonal cortisol antiserum coated onto the wells. After one-hour incubation the microplate is washed to stop the competition reaction. Wells are then incubated with the substrate tetramethylbenzidine (TMB). An acid stopping solution (sulphuric acid) is then added, and the optical density measured at 450nm. The concentration of salivary cortisol is inversely proportional to the optical density measured. A set of standards is used to plot a standard curve of absorbance versus salivary cortisol concentration from which the salivary cortisol concentrations in the unknowns can be calculated.

The Intra-assay (within each assay) variation was determined by replicate measurements of 4 saliva samples using DRG ELISA kit. The within variability is shown below:

Mean (ng/mL)	4.52	0.94	12.79	17.50
SD (ng/mL)	0.120	0.042	0.230	0.258
CV (%)	2.65	4.52	1.80	1.47
N=	20	20	20	20

The Inter-assay (between run) variation was determined by quadruplicate measurements of commercial control samples in three different days' runs:

Mean (ng/mL)	24.29	40.85
SD (ng/mL)	1.81	2.38
CV (%)	7.47	5.82
N=	12	12

The Inter-lot (between-lot) variation was determined by duplicate measurements of five saliva samples in three different kit lots. The between run variability is shown below:

Mean (ng/mL)	1.22	12.65	15.8	4.16	4.53
SD (ng/mL)	0.07	0.35	0.70	0.10	0.12
CV (%)	5.97	2.73	4.43	2.35	2.72
N=	9	9	9	9	9

As far as the sensitivity is concerned, the lowest detectable level of cortisol that can be distinguished from the zero standard is 0.537 ng/mL or 0.0357 µg/dl at the 95% confidence limit.

IV.11 Cognitive Function tests, Version 2

Only the different tests with regard to Version 1 are going to be presented, that is Word list, Matrices, and Number Search test.

Word list

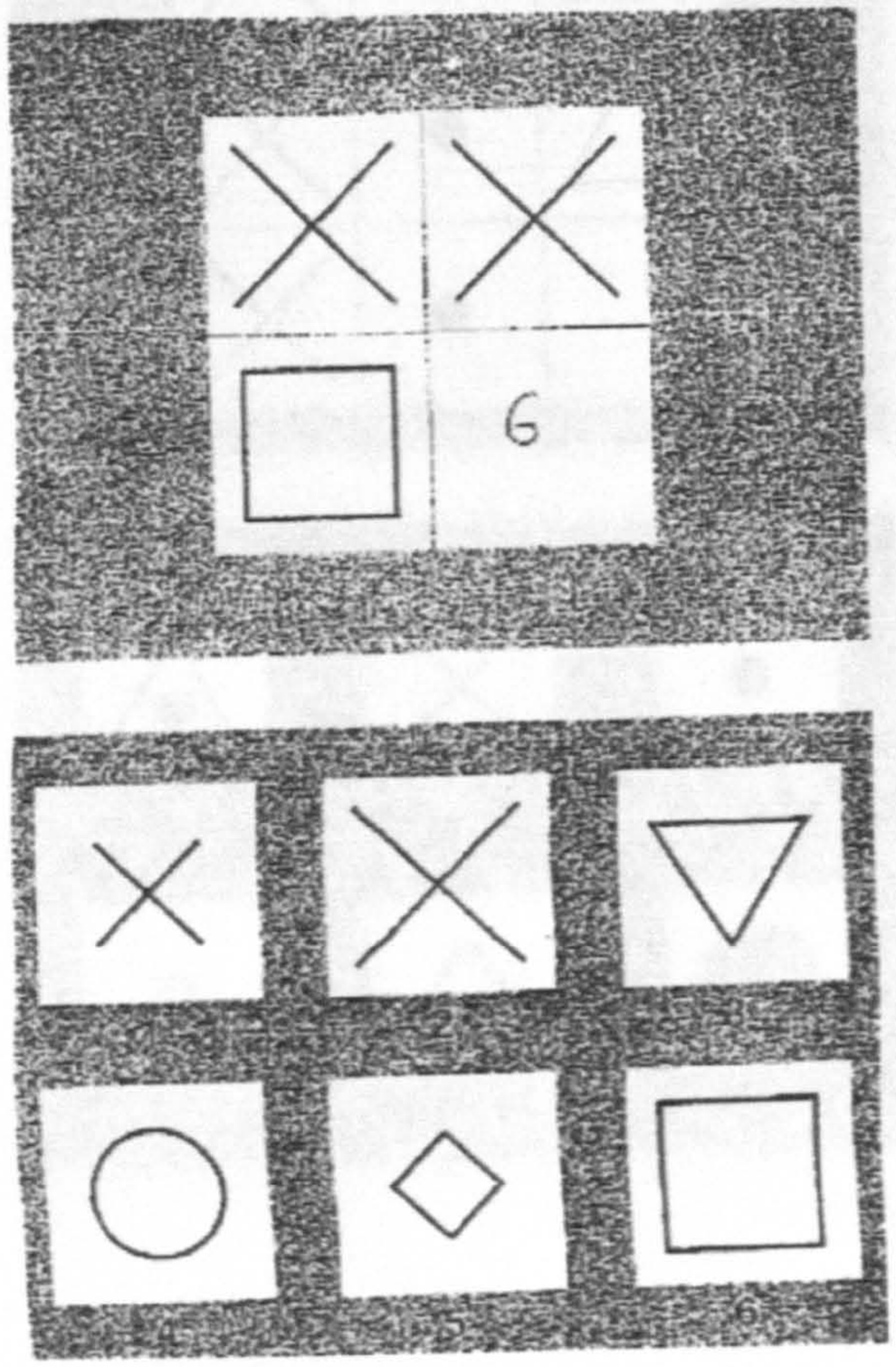
Study carefully the list of 15 words. Don't write anything down.

Item
Series
Advice
Custom
Owner
Circuit
Humour
Nephew
Safety
Vessel
Code
Fortune
Link
Boss
Shadow

Do NOT turn over to the next page yet

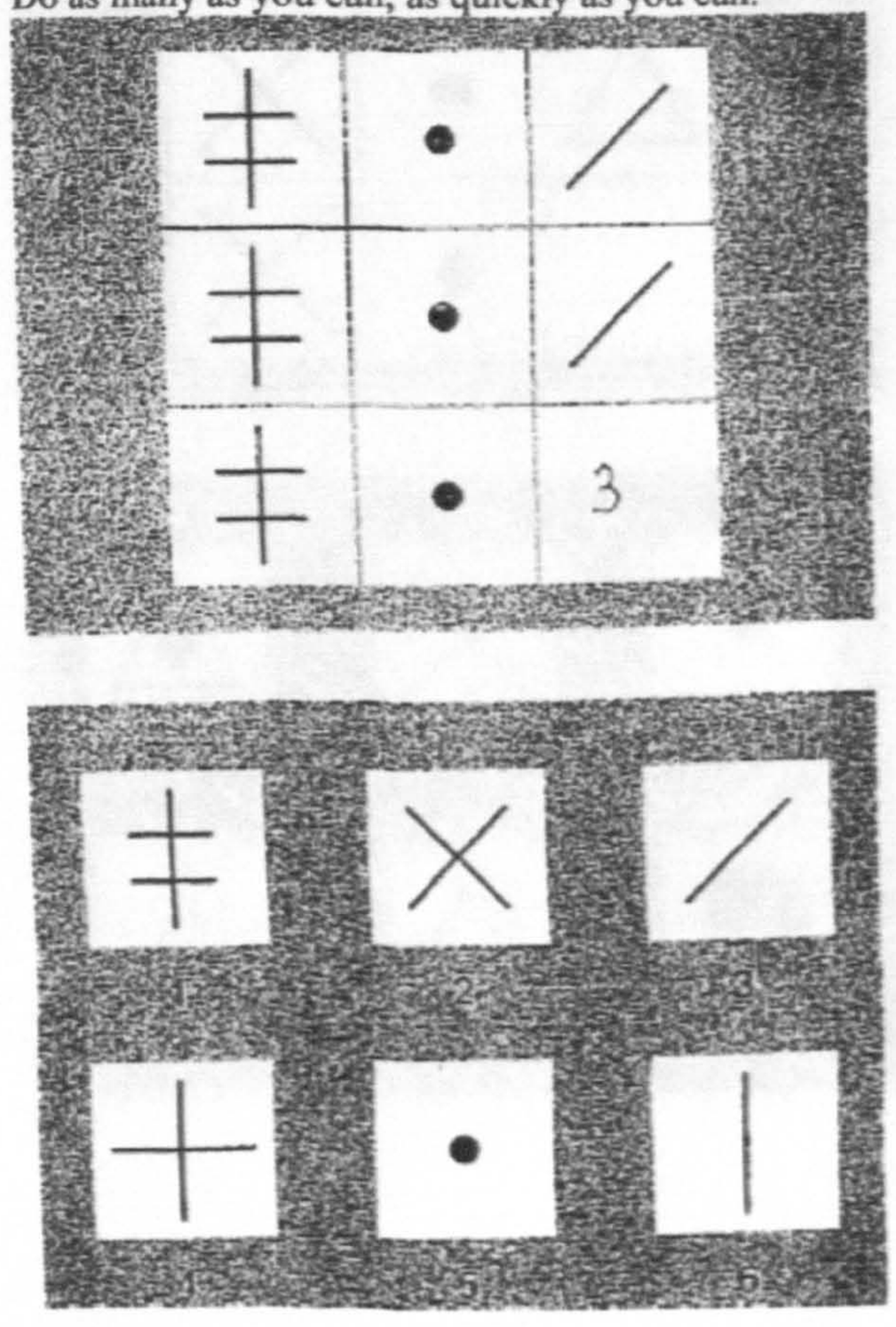
Find the missing shape

Write the number of the missing shape in the empty box, like this

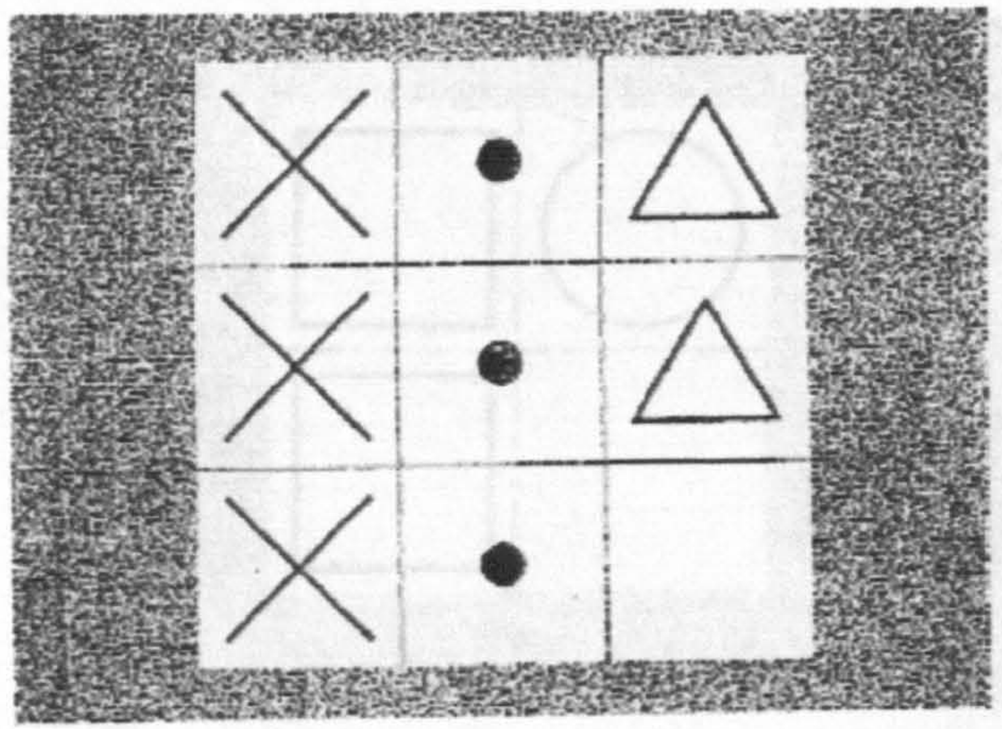


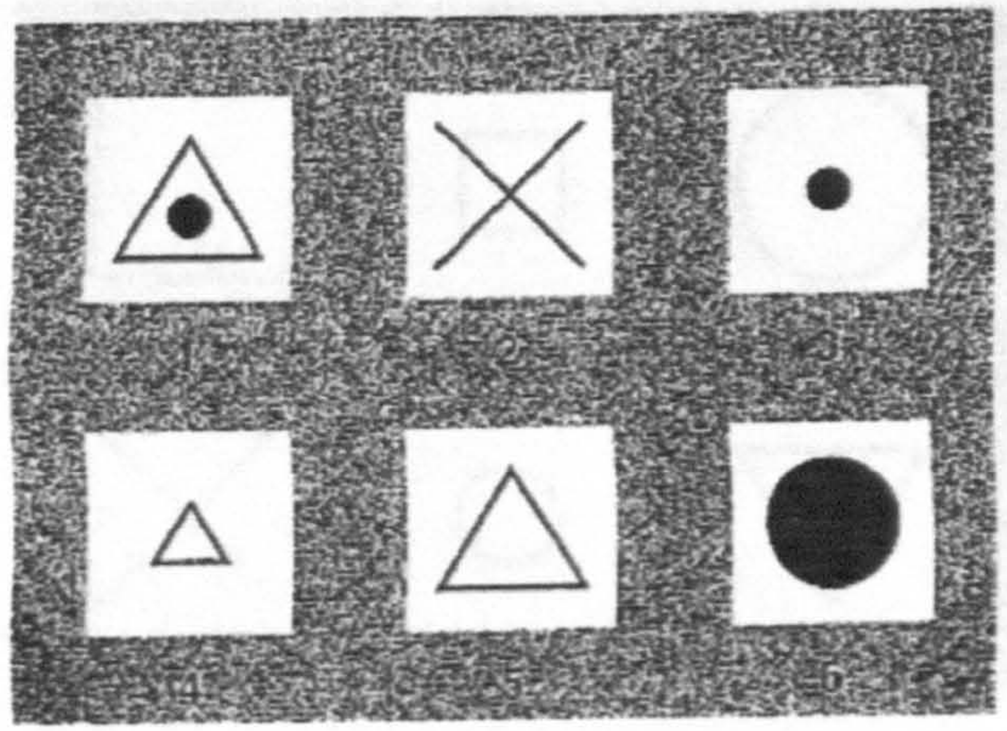
and like this

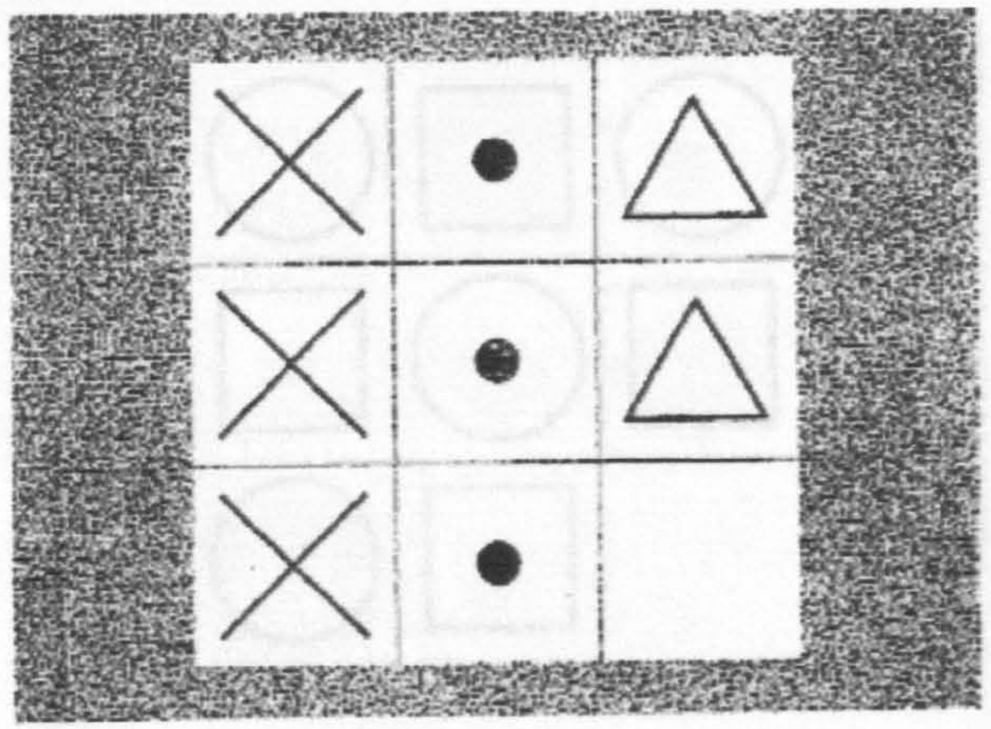
On the next 16 pages there are more of these puzzles.
They gradually get harder
Do as many as you can, as quickly as you can.

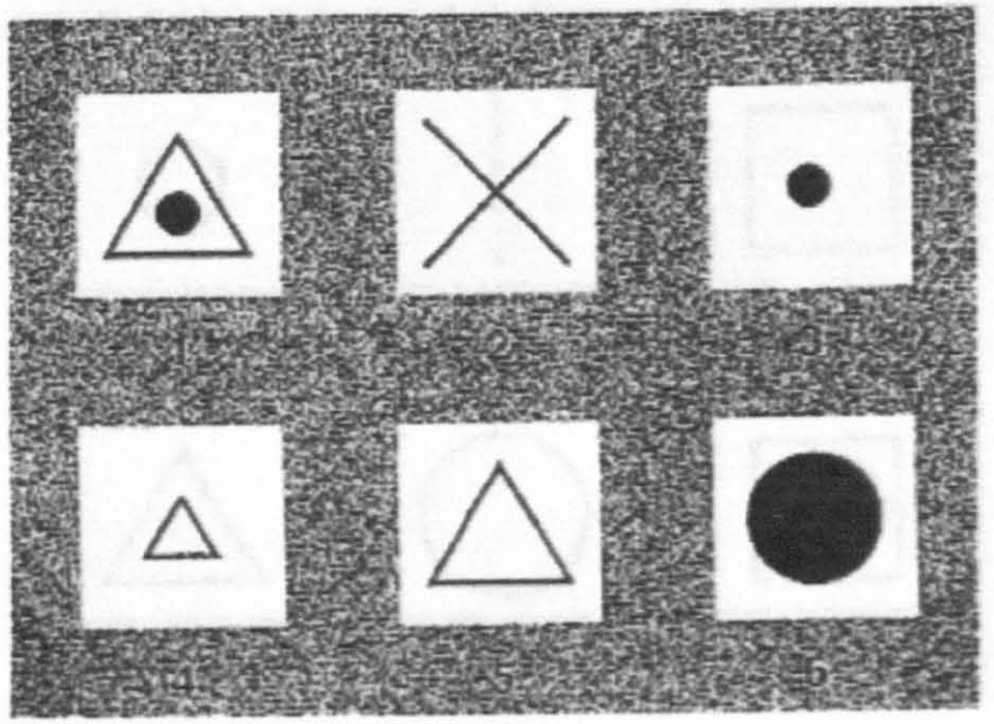


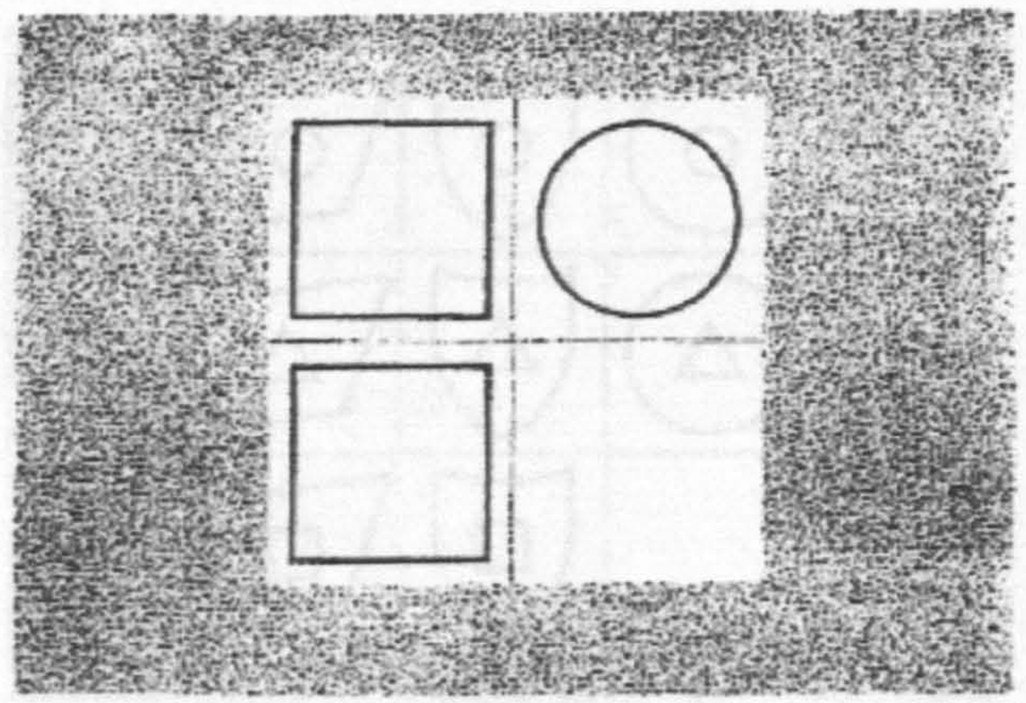
Do NOT turn over to the next page yet

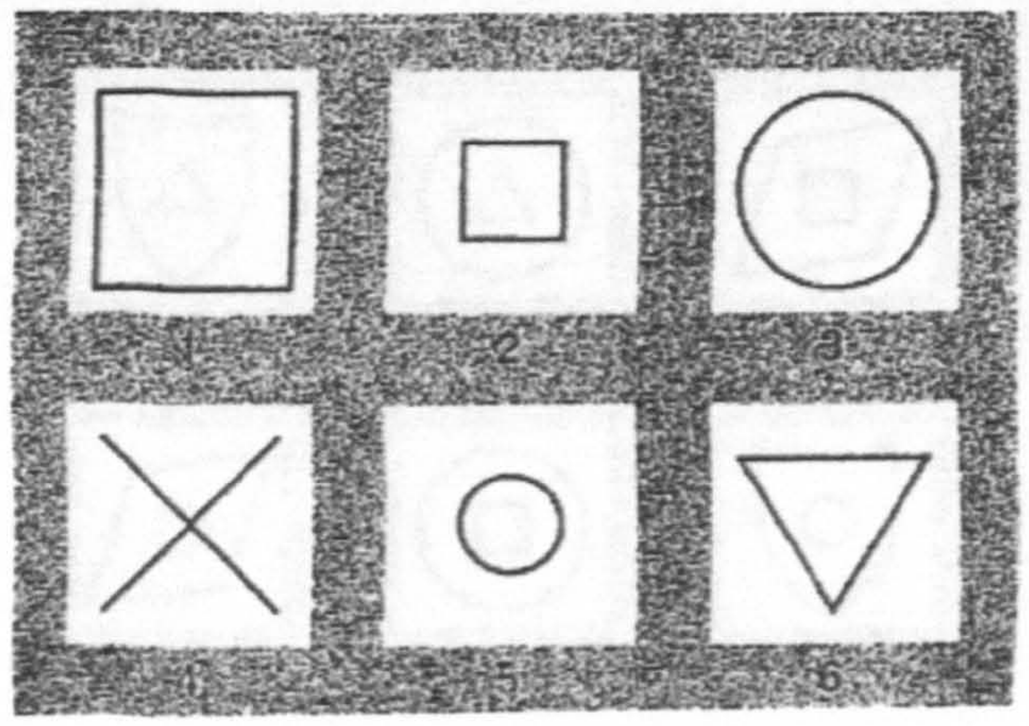


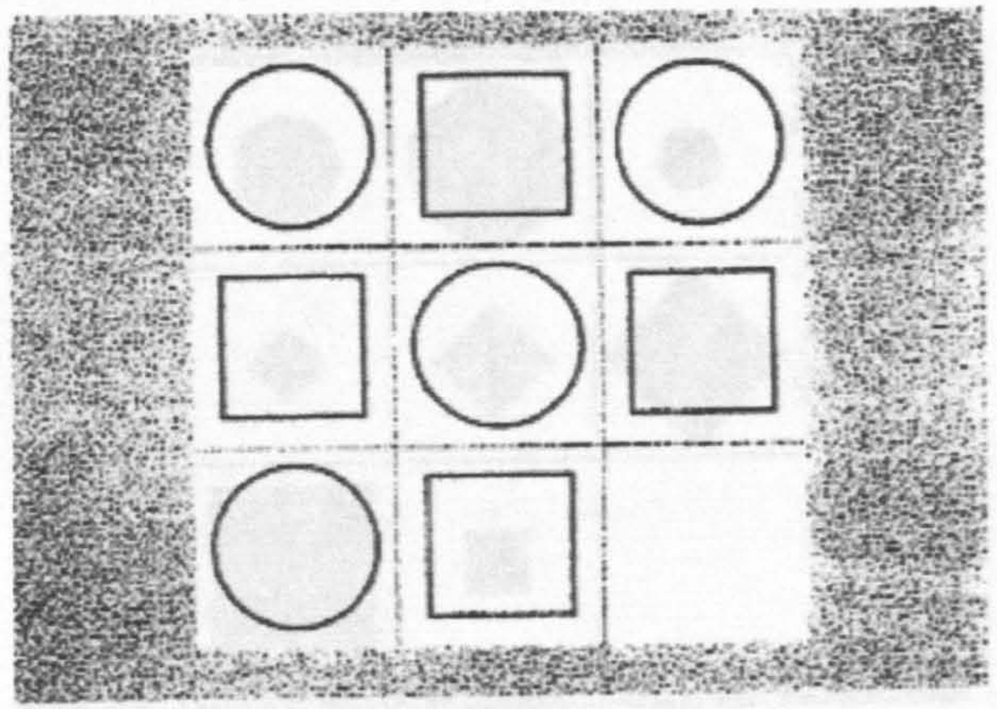


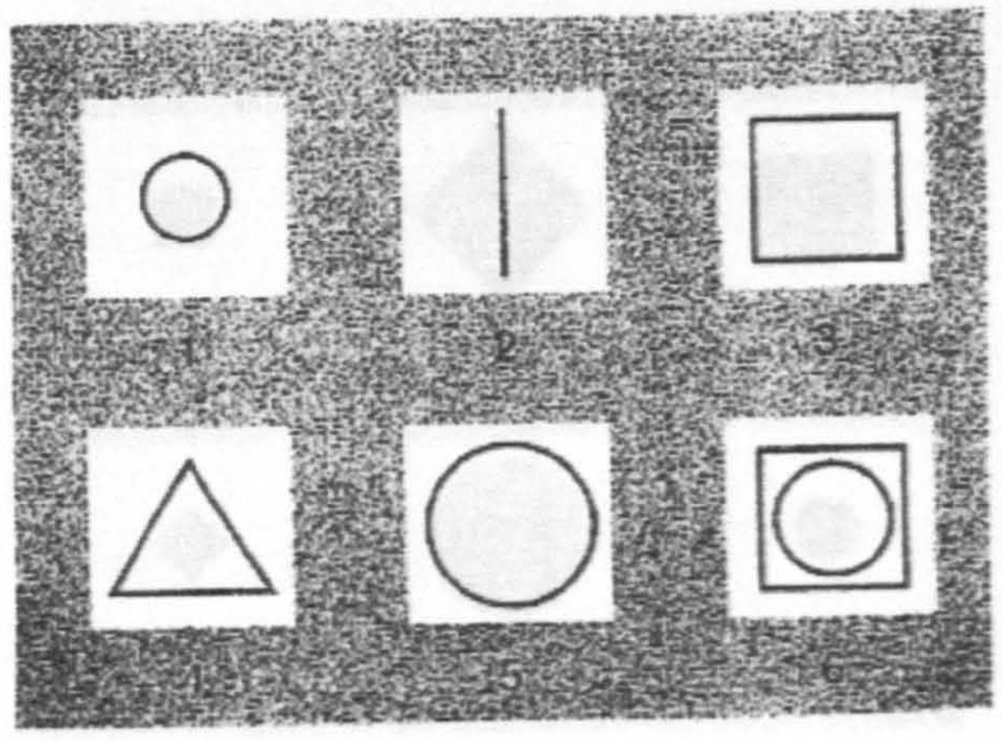


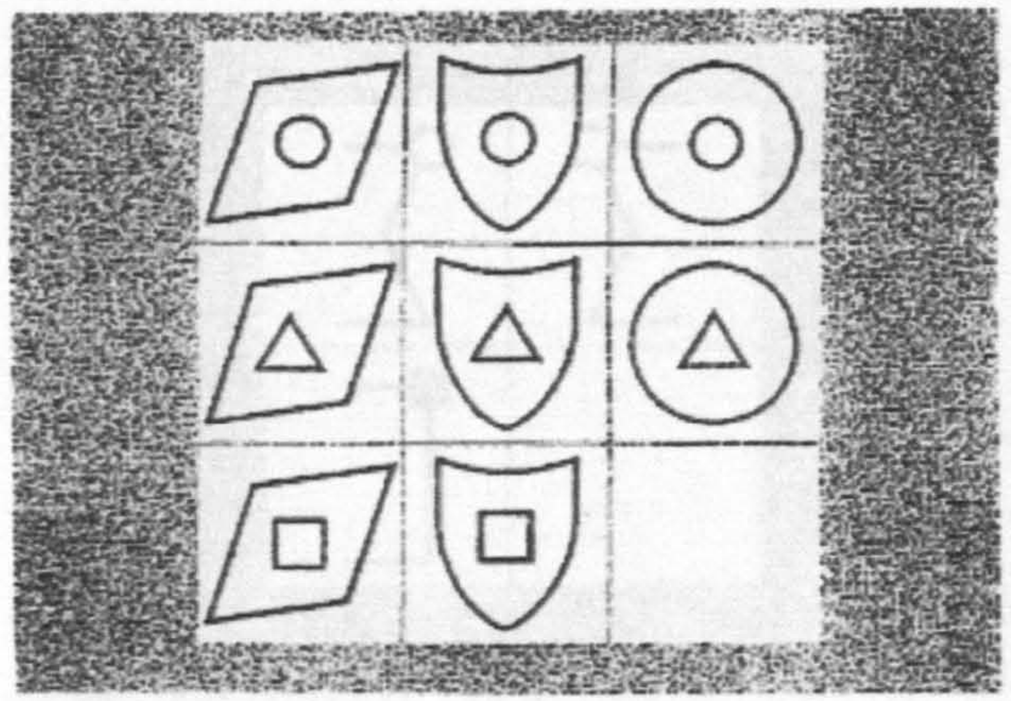


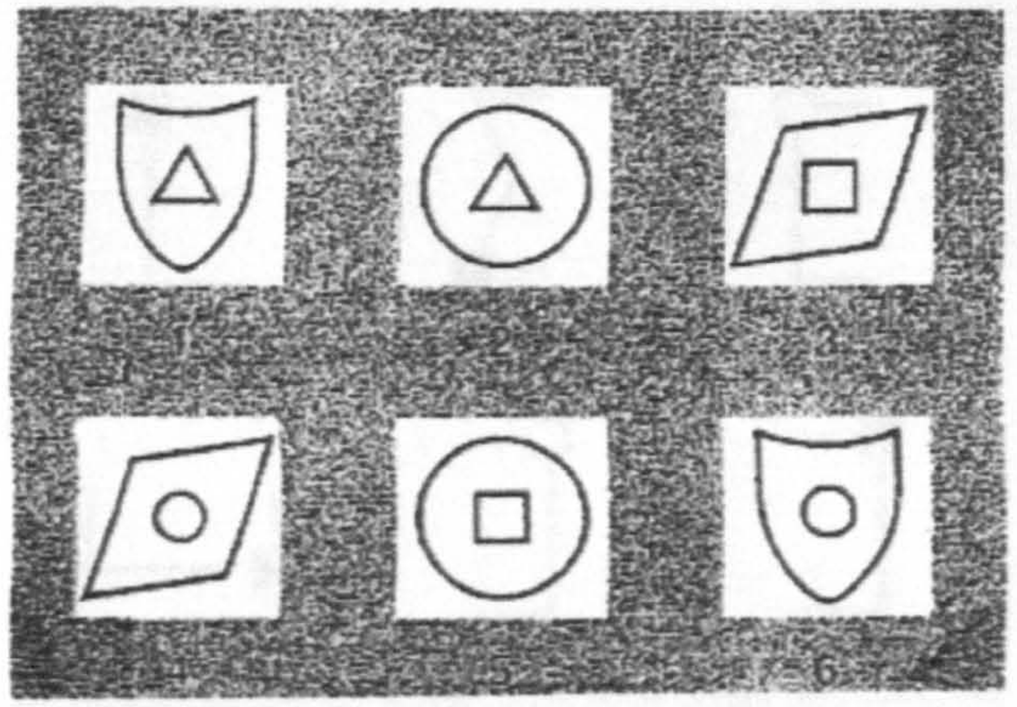


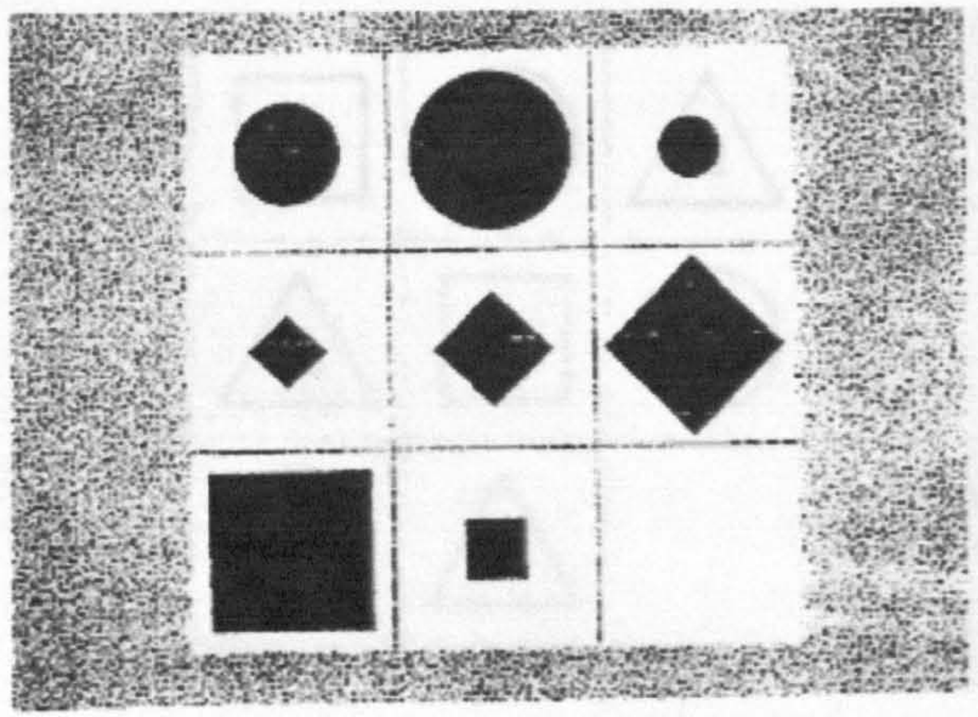


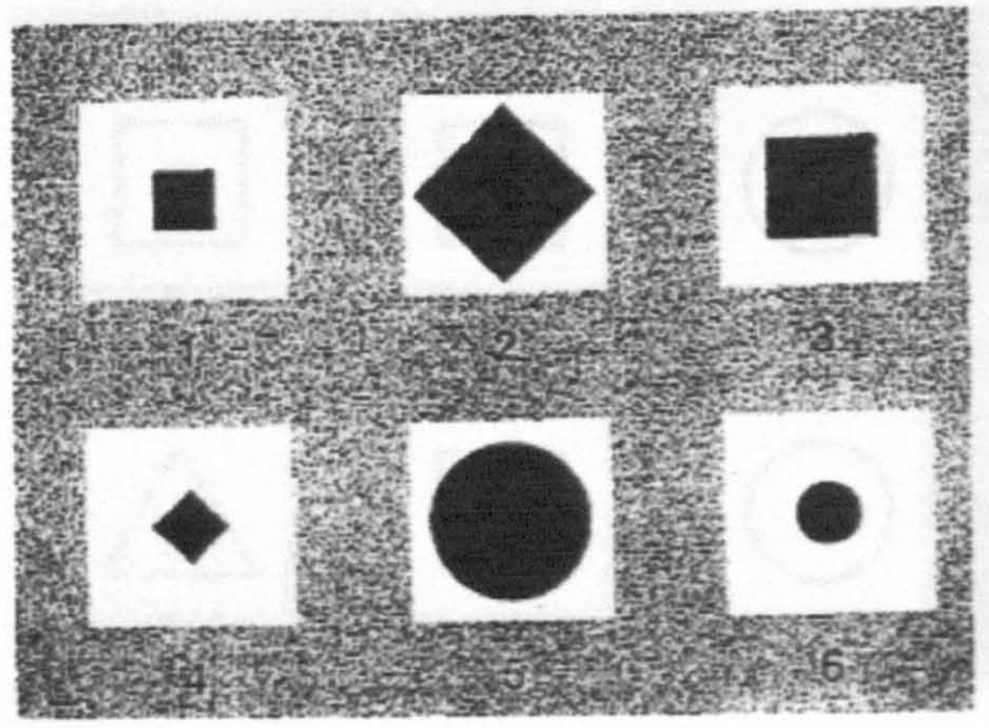


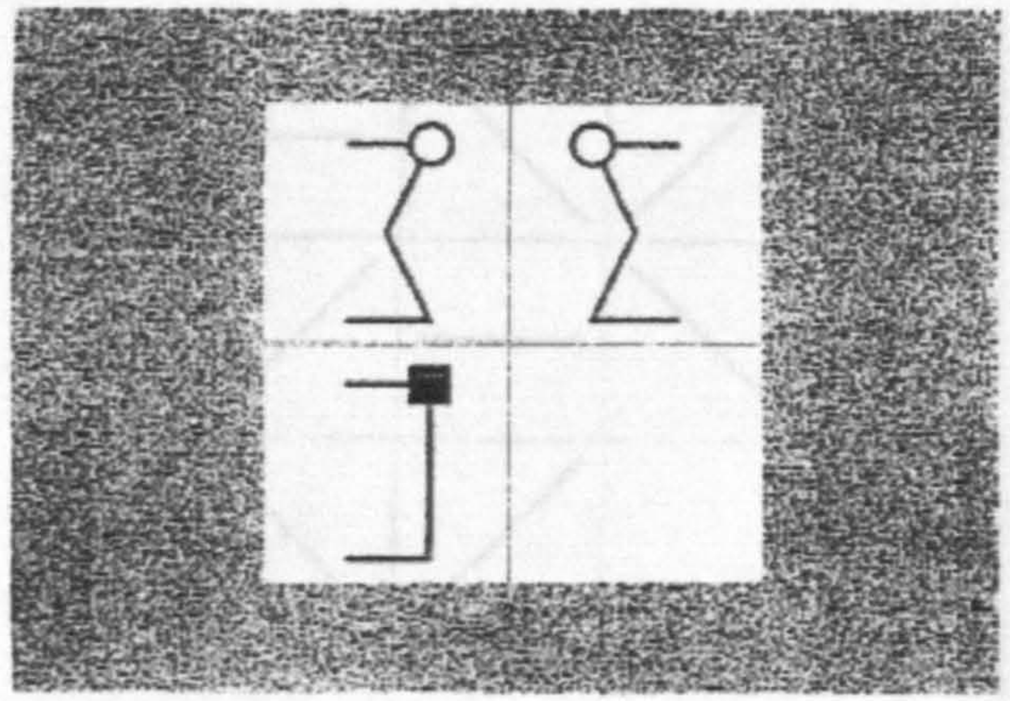


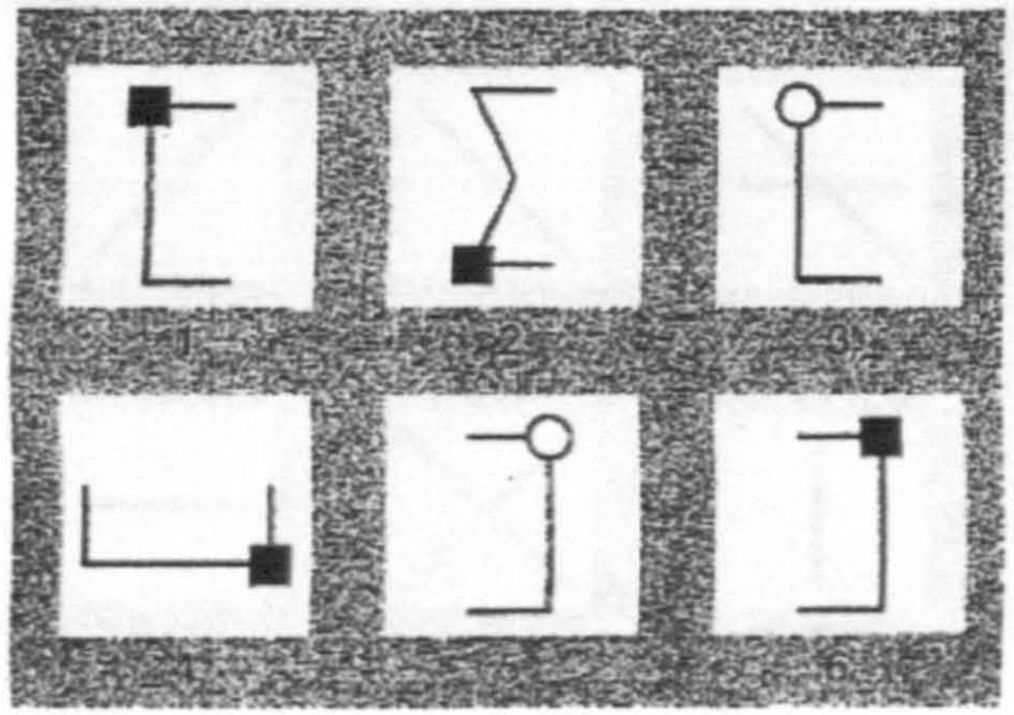


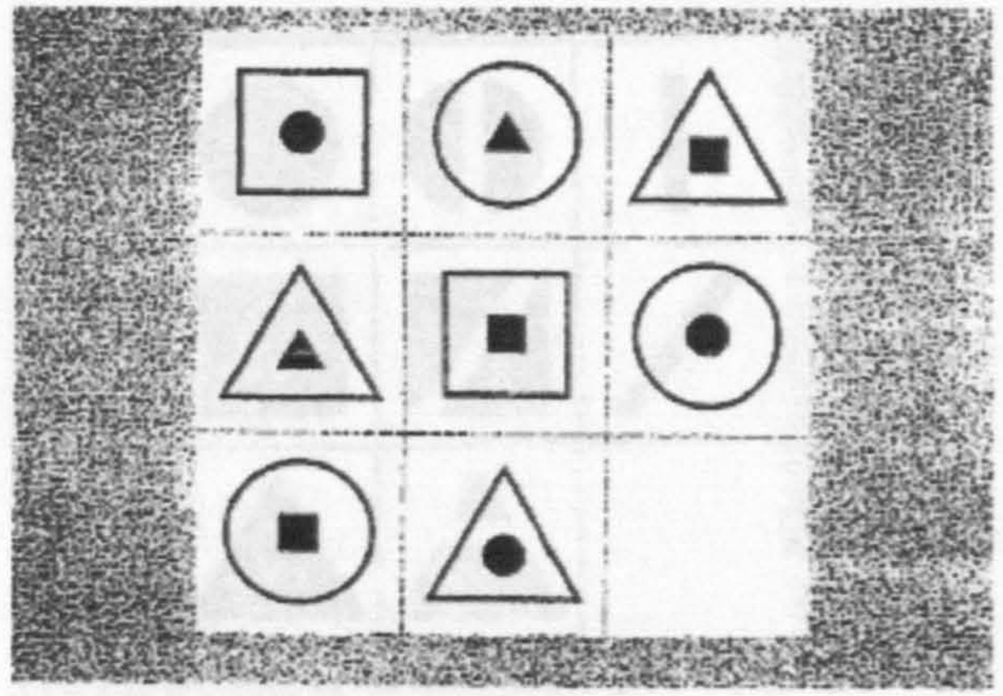


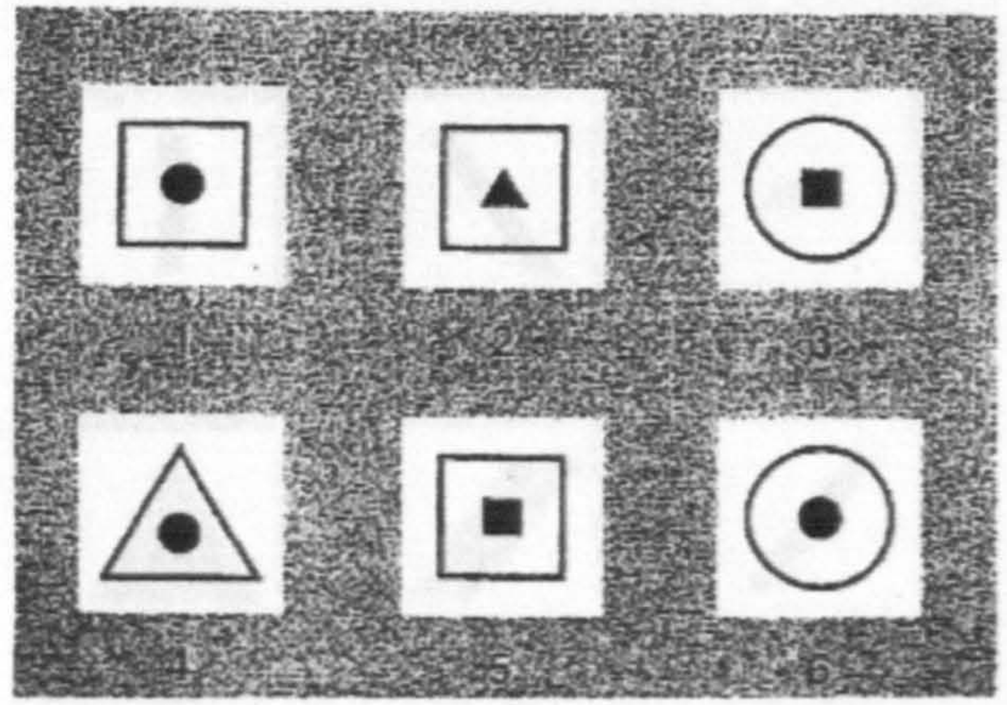


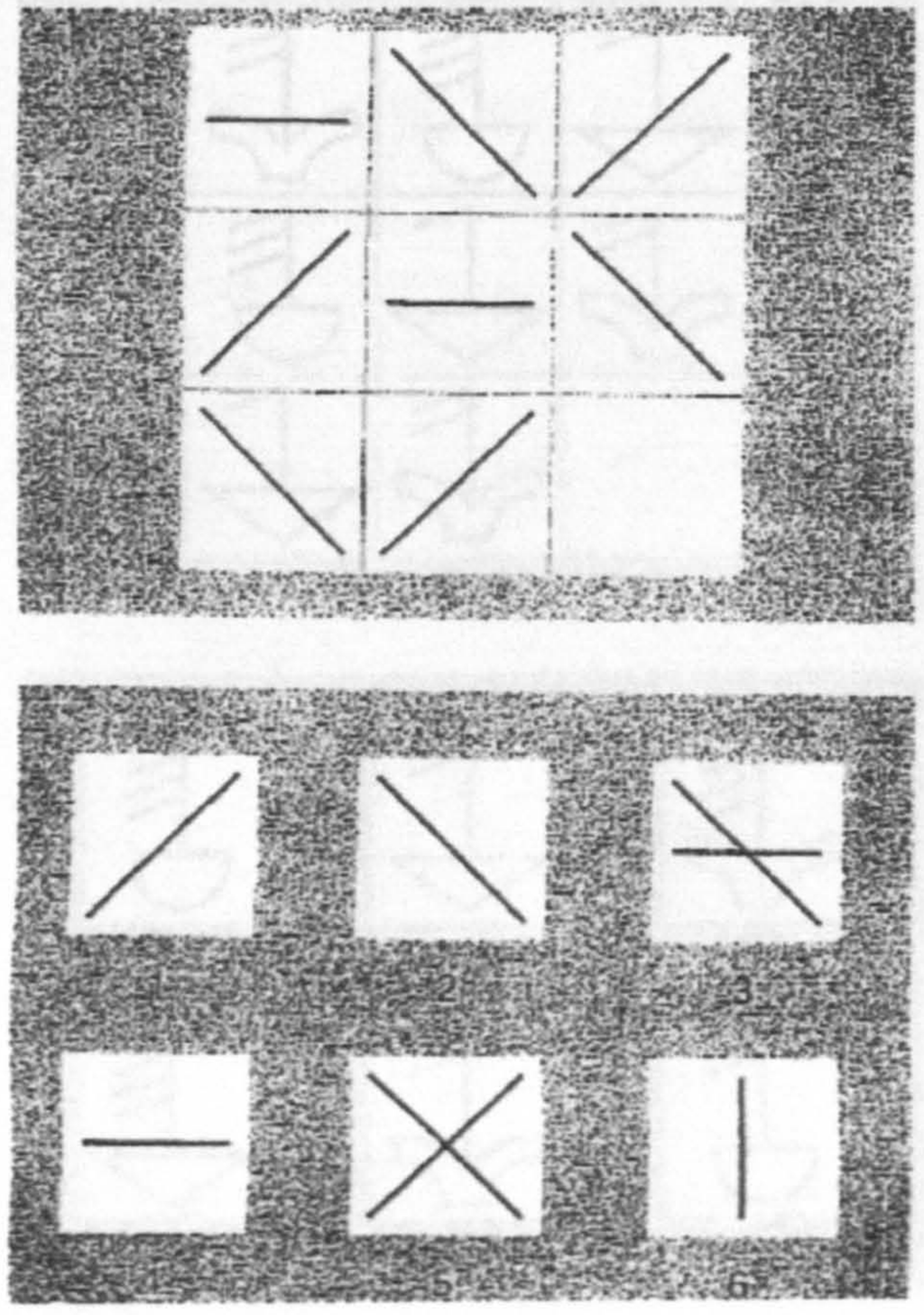


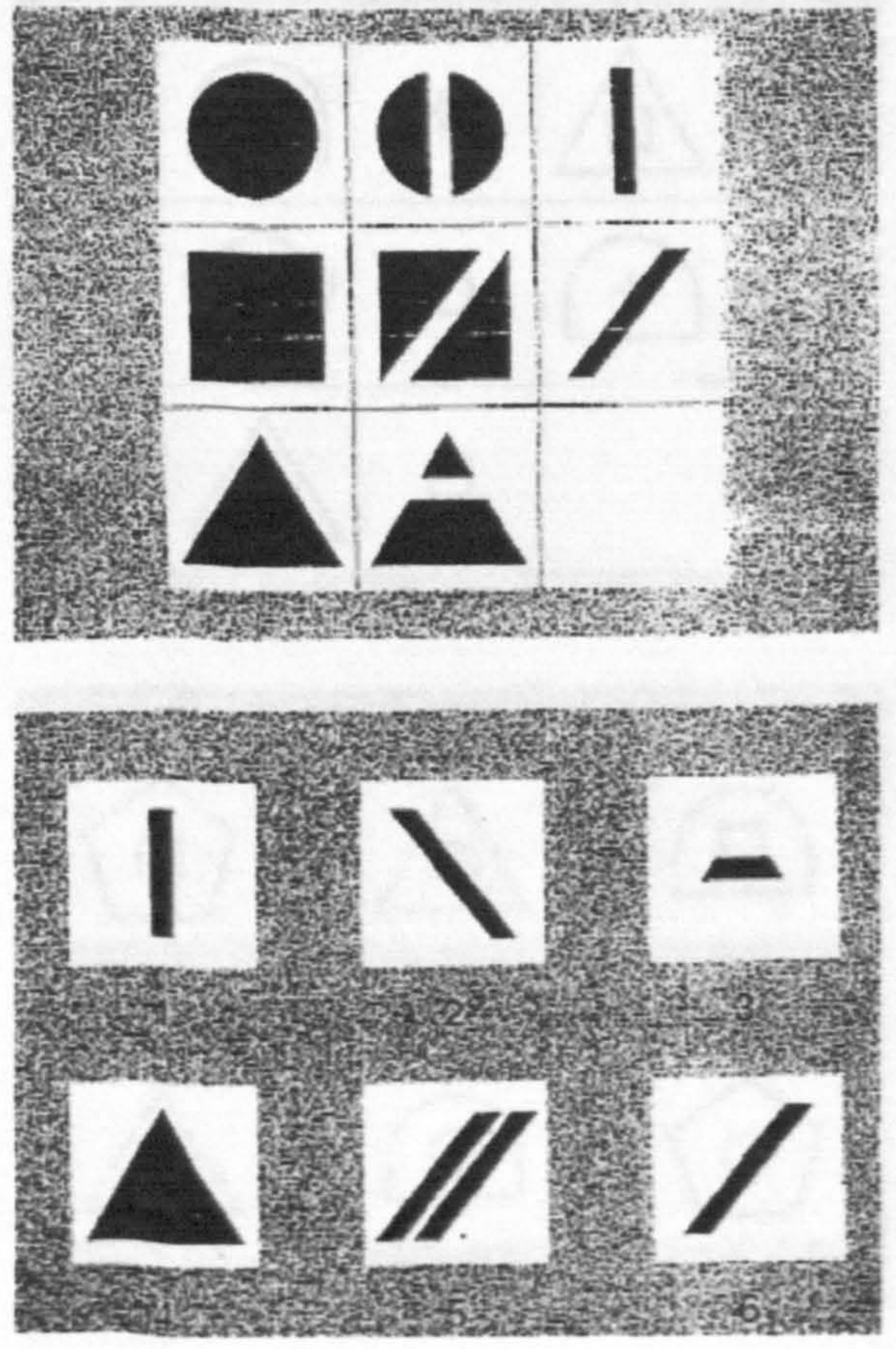


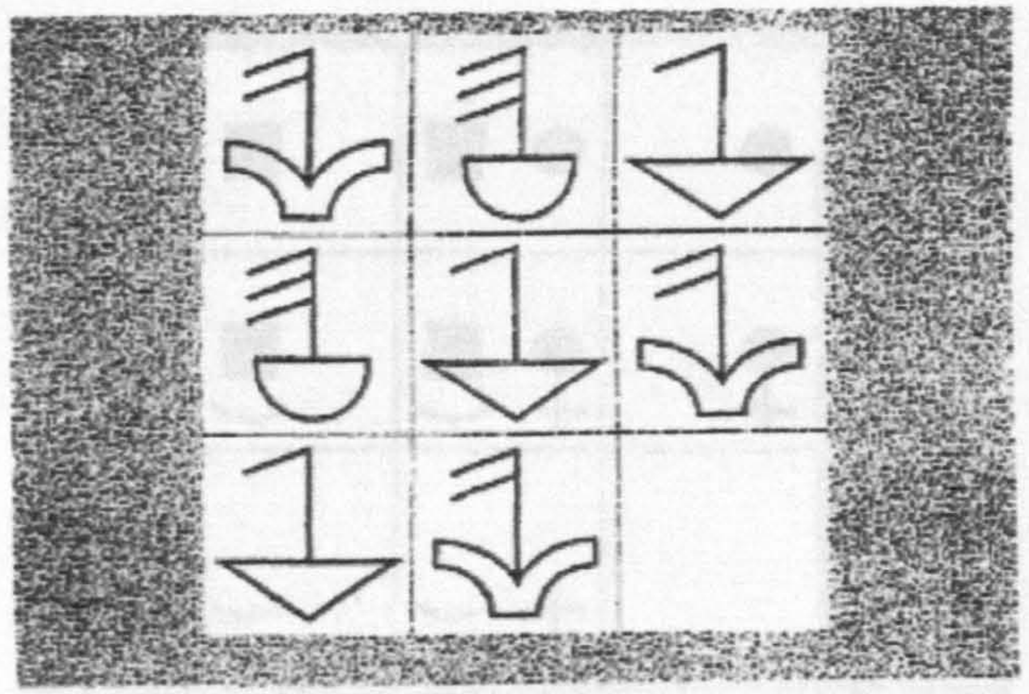


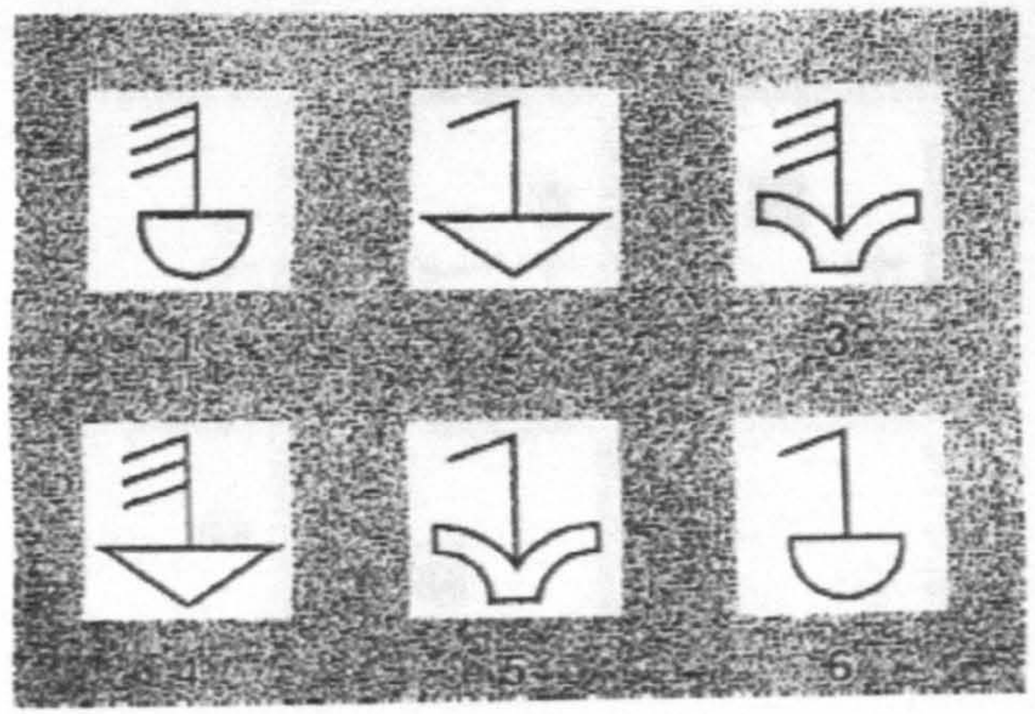








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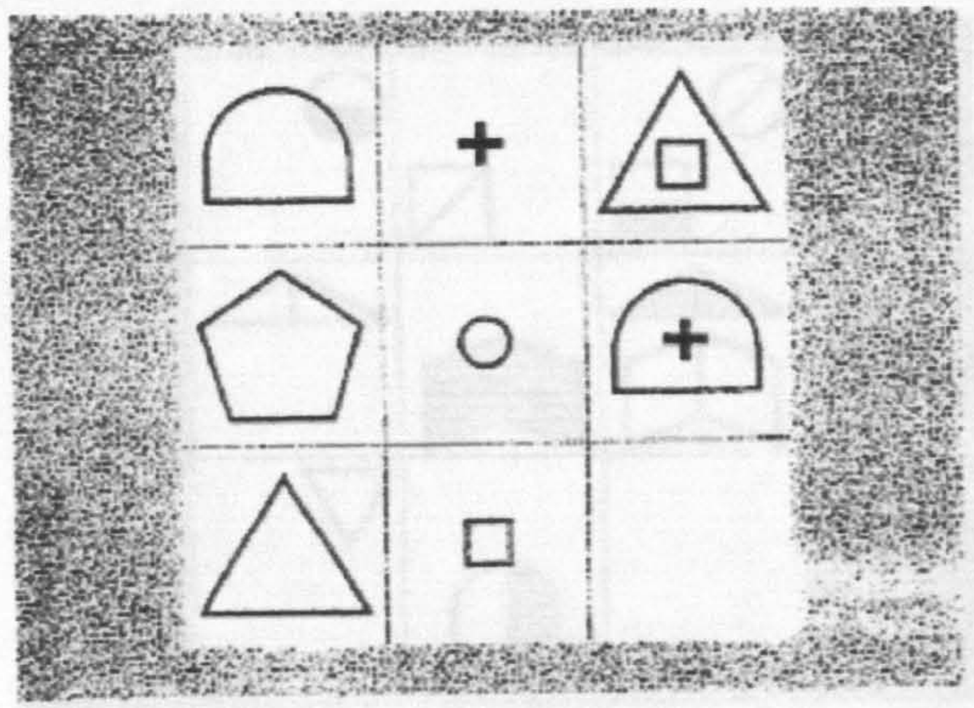

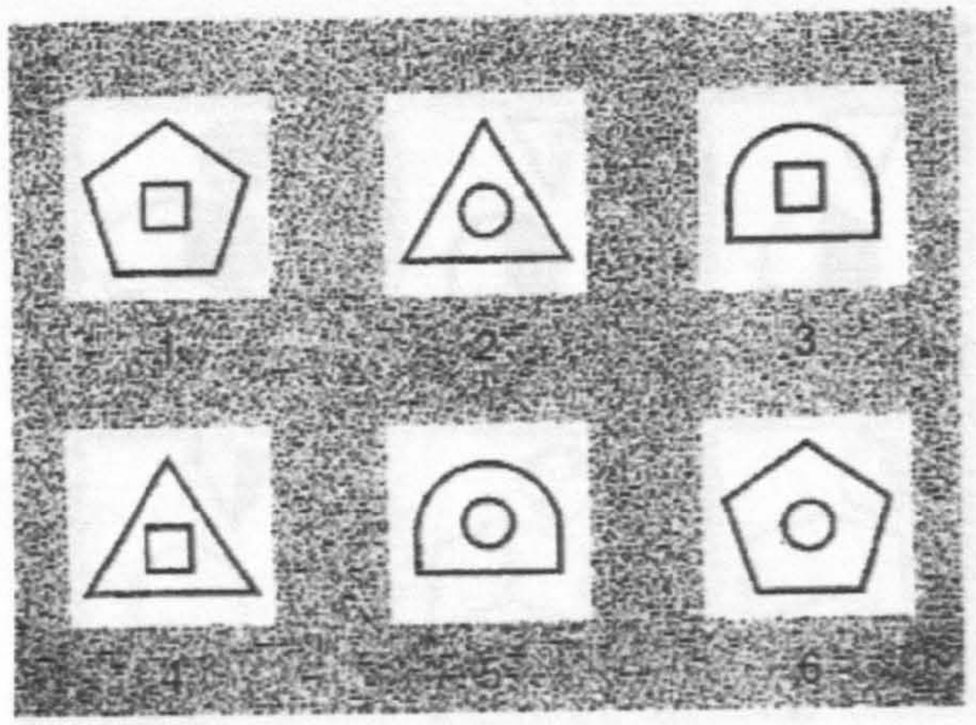
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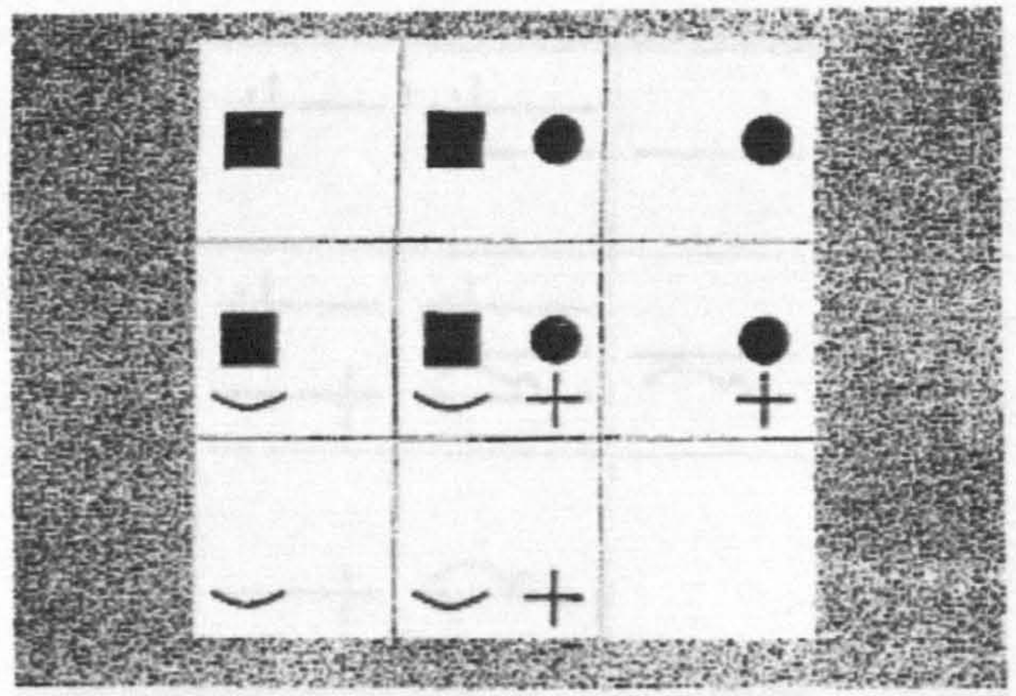

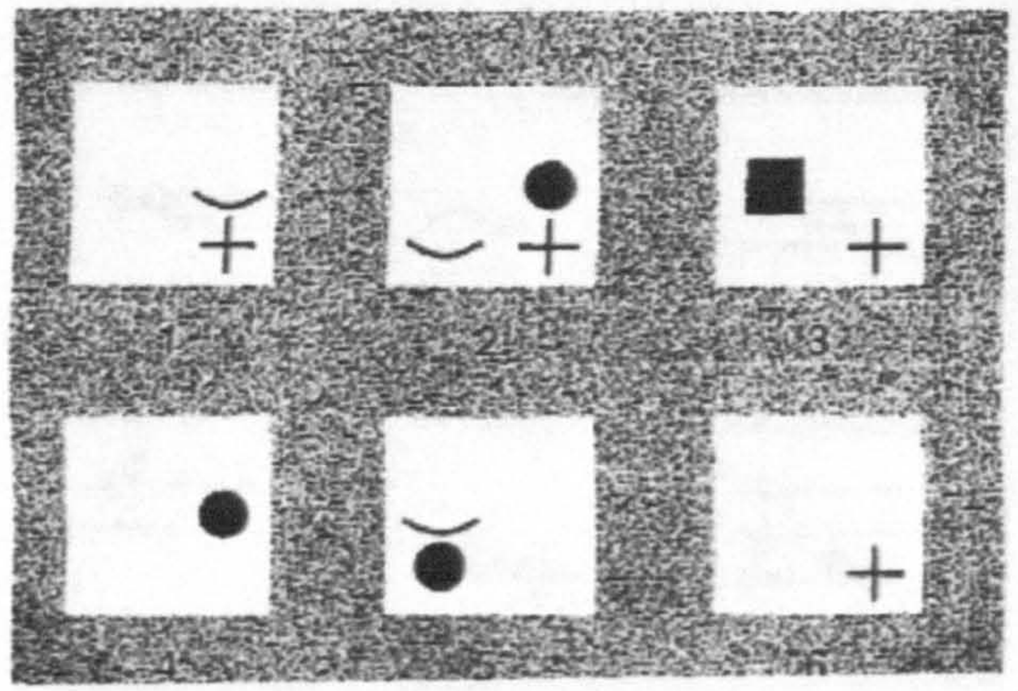

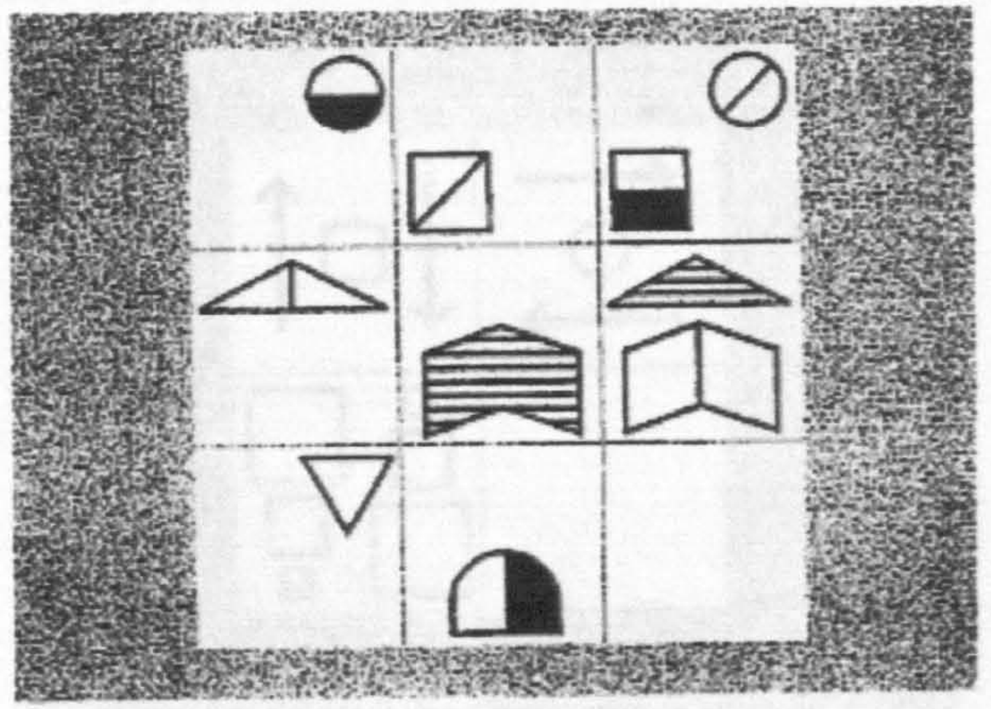

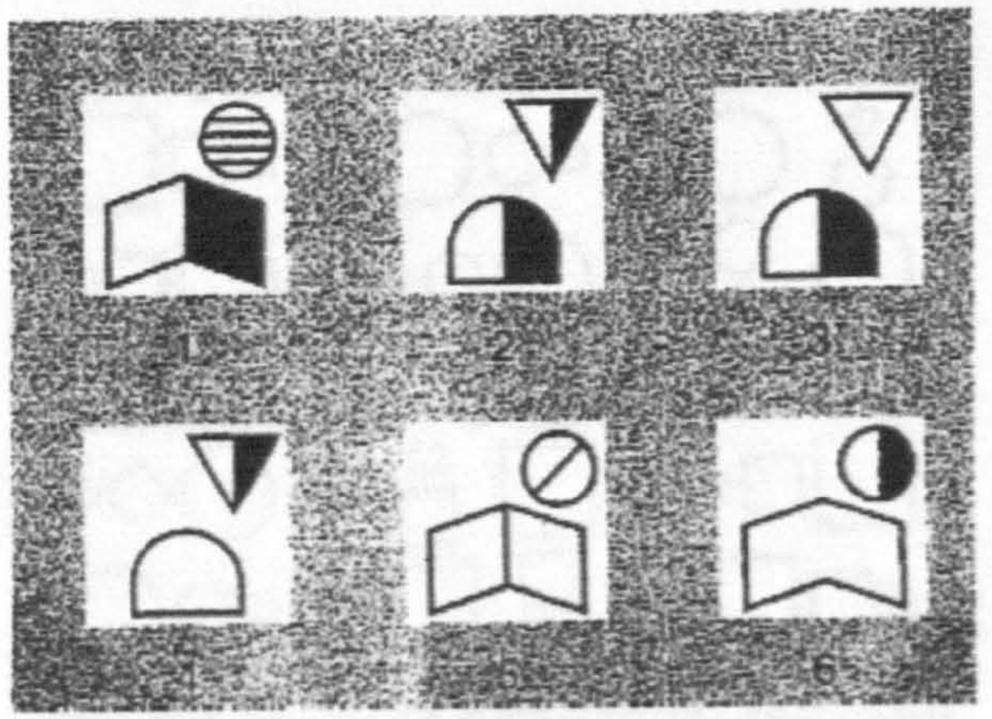

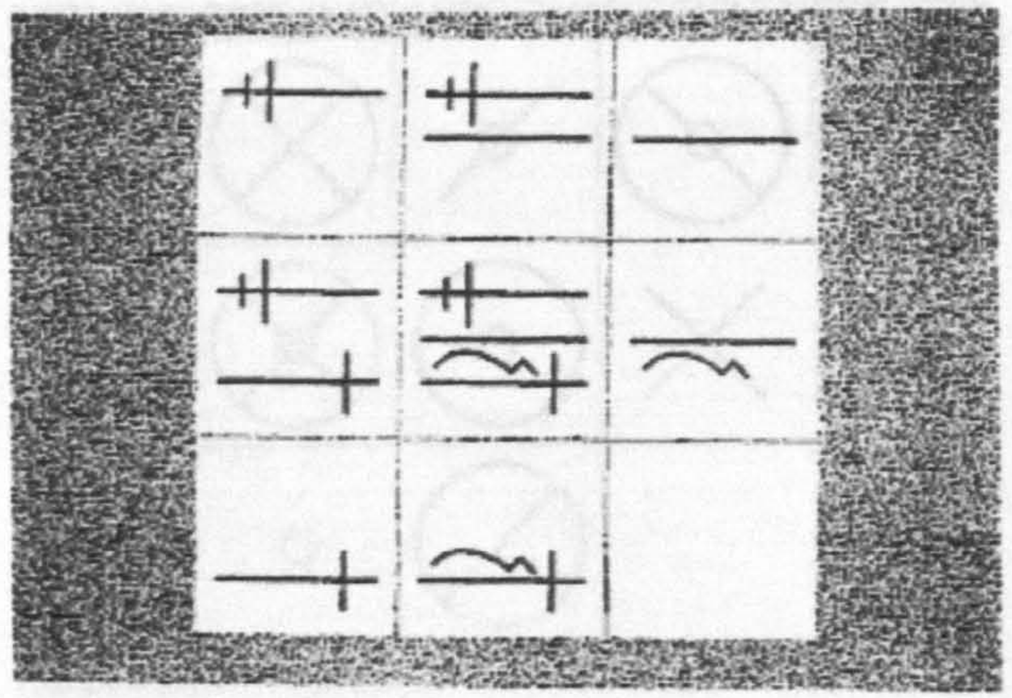

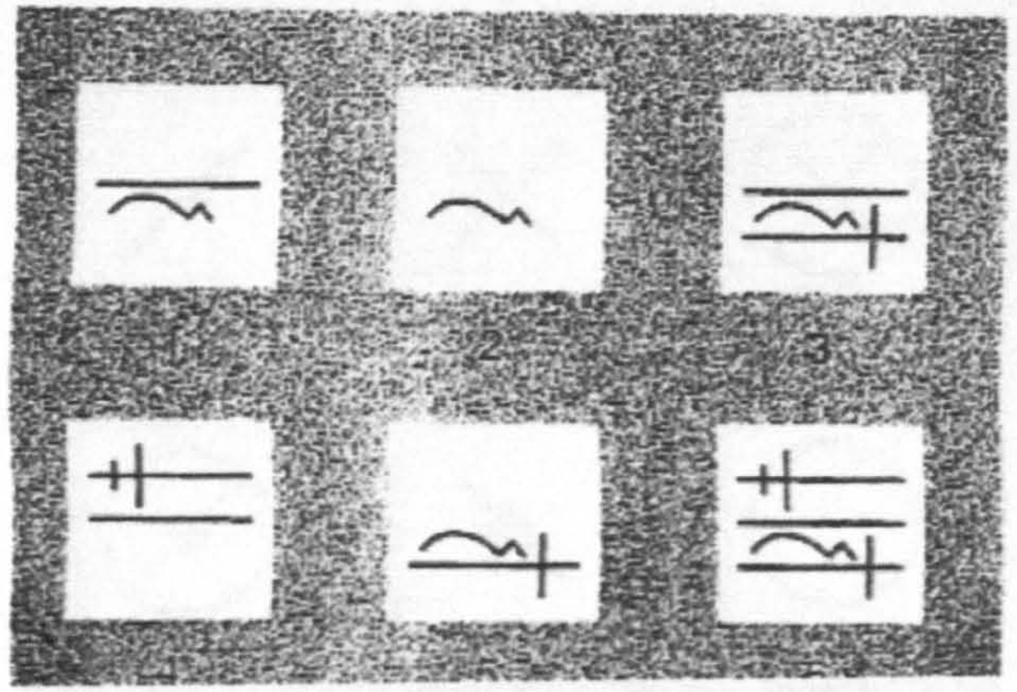

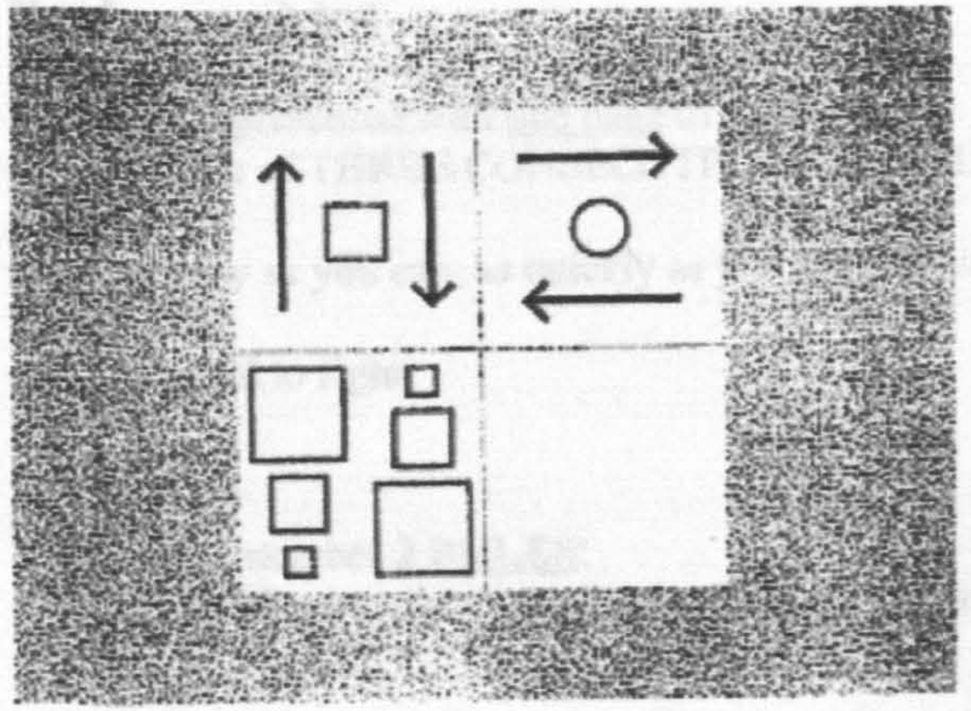

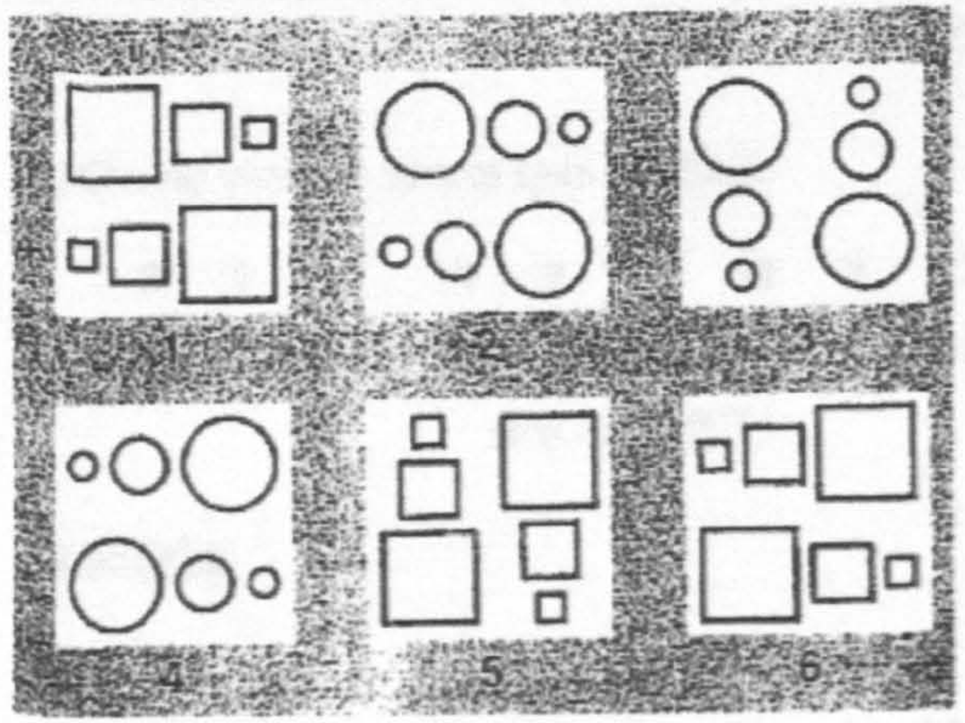

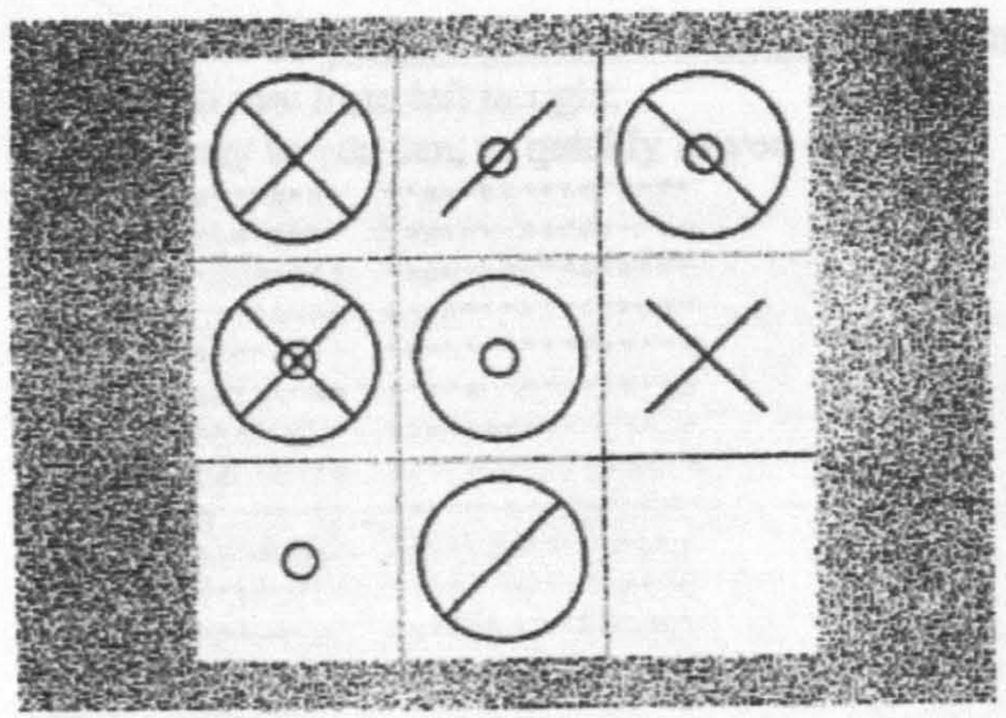

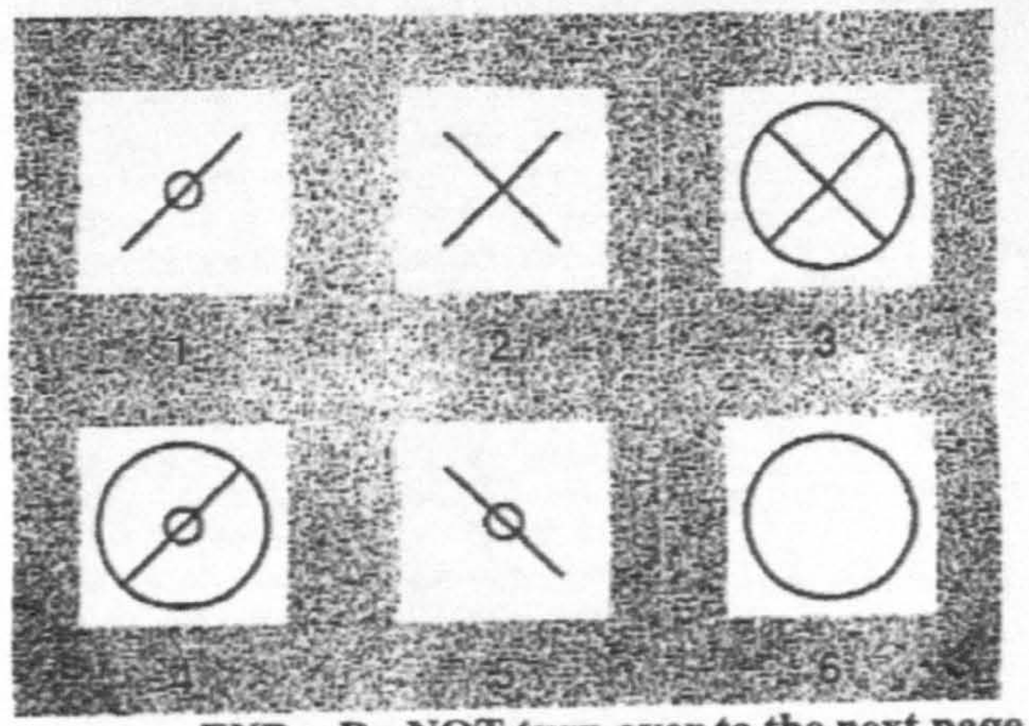

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Number search test

You will be presented with one page of numbers.
Circle blocks of THREE CONSECUTIVE EVEN NUMBERS.

Find as many as you can, as quickly as you can. Move across each row, from left to right.

You must remember **2 RULES**:
(1) Each number is only allowed in one line of circled numbers

4 12 8 5 7 6 6 14 12 2
(CORRECT) (INCORRECT)

(2) Circled numbers cannot span two lines

8 5 13 14 9 2 10 5 12 6
4 17 15 14 10 13 3 6 11 7
(INCORRECT)

EXAMPLE

13 2 6 8 9 5 14 5 9 11
14 5 6 12 10 5 15 4 14 16

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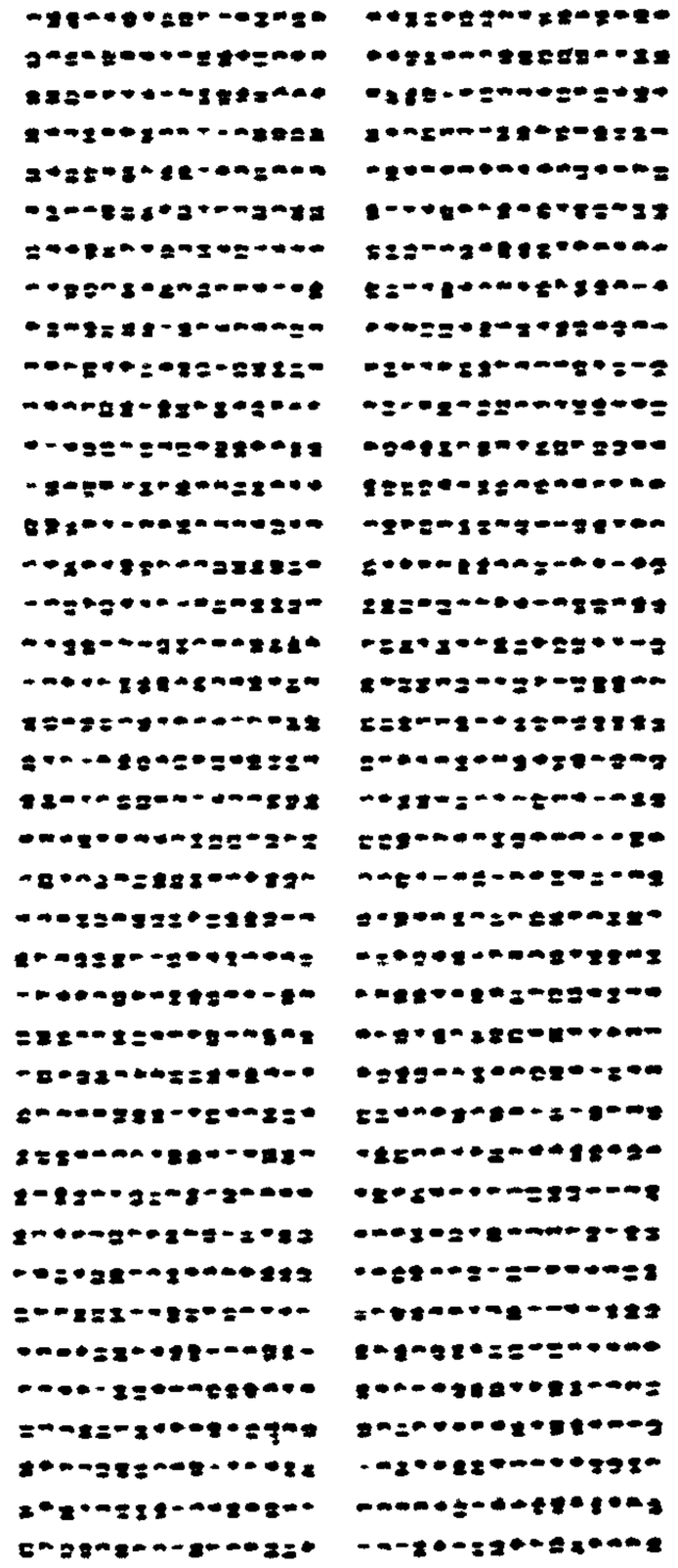
Circle blocks of THREE CONSECUTIVE EVEN numbers, moving across each row from left to right.
Find as many as you can, as quickly as you can.



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IV.12 Immediate and delayed memory task: information on the list of words used

Information regarding the list of words used in Version 1 is presented in section II.9, page 311). Therefore, information regarding the list of words in Version 2 is presented here:
This same website was used as for Version 1:
http://www.math.yorku.ca/SCS/Online/paivio/

Version 2	Frequency	Imagery	Concreteness	Meaning-fulness	Number of syllables	Number of letters
item	26	3.67	5.27	4.96	2	4
series	50	4.47	3.88	5.36	2	6
advice	50	3.13	2.08	5.39	2	6
custom	50	3.43	2.99	5.33	2	6
owner	50	4.23	5.9	5.71	2	5
circuit	21	4.97	5.63	6.38	2	7
humour	41	4.57	2.31	5.72	2	6
nephew	22	4.3	6.19	4.48	2	6
safety	50	4.27	2.25	5.76	2	6
vessel	50	5.9	6.99	7.82	2	6
code	21	4.53	4.53	6.63	1	4
fortune	50	4.57	3.82	6	2	7
link	24	4.8	5.38	5.2	1	4
boss	23	5.73	6.35	5.81	1	4
shadow	50	5.63	4.94	5.28	2	6
Total	**38.53**	**4.55**	**4.57**	**5.72**	**1.80**	**5.53**

IV.13 Nutrient Databank

The nutrient databank and details of nutrients measured
Intakes of nutrients were calculated from the records of food consumption using a specially adapted nutrient databank. The nutrient databank was originally developed for the Ministry of Agriculture, Fisheries and Food (MAFF) for the Dietary and Nutritional Survey of British Adults. It was updated for the National Diet and Nutrition Surveys (NDNS) of children aged 1½-4½ years, people aged 65 years and over, and young people aged 4-18 years. Further revisions and updates were carried out by the Food Standards Agency (FSA) for the NDNS of adults aged 19 to 64 years. It was revised again by nutritionists at King's College London (KCL) for the Low Income Diet and Nutrition Survey (LIDNS). Relatively few updates were made for this survey; those that were made were mainly for additional homemade recipes.

The databank contains nutritional information on over 8,000 foods and drinks, including manufactured products, homemade recipe dishes and many types of dietary supplements. Each food on the databank has values assigned for 54 nutrients and energy. The nutrient values assigned to the foods in the databank are based on data from the Agency's rolling programme of nutrient analysis of foods. These data are also incorporated into McCance and Widdowson's The Composition of Foods series. New analytical values were incorporated for LIDNS, along with data from the Agency's (2004) 'catch-up' project, which analysed composite samples of a wide range of foods for which the Agency did not have detailed information.

Details of nutrients measured and units

Nutrient	Units
Water	(g)
Sugars	(g) total sugars, expressed as monosaccharide
Starch	(g) expressed as monosaccharide
non-starch polysaccharides	(g) expressed as Englyst method
energy	(kJ) (17 x protein) + (37 x fat) + (16 x carbohydrate) + (29 x alcohol)
Energy	(kcal) (4 x protein) + (9 x fat) + (3.75 x carbohydrate) + (7 x alcohol)
protein	(g)
nitrogen	(g)
fat	(g)
carbohydrate	(g) sum of sugars plus starch, expressed as monosaccharide equivalent
Alcohol	(g)
Sodium	(mg)
potassium	(mg)
calcium	(mg)
magnesium	(mg)
phosphorus	(mg)
iron	(mg)
haem iron	(mg)
non-haem iron	(mg)
copper	(mg)
zinc	(mg)
Chloride	(mg)
iodine	(µg)
manganese	(mg)
retinol	(µg) all *trans* retinol equivalents
total carotene	(µg) ß carotene equivalents
α-carotene	(µg)
ß-carotene	(µg)
ß-cryptoxanthin	(µg)
thiamin	(mg)
riboflavin	(mg)
niacin equivalent	(mg) niacin + (tryptophan / 60)
vitamin B6	(mg)
vitamin B12	(µg)
folate	(µg)

pantothenic acid	(mg)
biotin	(µg)
vitamin C	(mg)
vitamin D	(µg)
vitamin E	(mg) α-tocopherol equivalents
fatty acids	
saturated	(g)
cis monounsaturated	(g)
cis n-3 polyunsaturated	(g)
cis n-6 polyunsaturated	(g)
trans fatty acids	(g)
cholesterol	(mg)
Sugars	
Glucose	(g)
Sucrose	(g)
Fructose	(g)
Lactose	(g)
Maltose	(g)
other sugars	(g) includes oligosaccharides
non-milk extrinsic sugars	(g) includes all sugars in fruit juices, table sugar, honey, sucrose, glucose and glucose syrups added to food + 50% of the sugars in canned, stewed, dried or preserved fruits
Intrinsic and milk sugars	(g) includes all sugars in fresh fruit and vegetables + 50% of the sugars in canned, stewed, dried or preserved fruits + lactose in milk.

REFERENCES

1. Abercrombie HC, Kalin NH, Thurow ME, Rosenkranz MA & Davidson RJ (2003) Cortisol variation in humans affects memory for emotionally laden and neutral information. *Behav Neurosci* **117**, 505-516.

2. ADA (1996) Consensus statement on Clinical Practice Recommendation: self-monitoring of blood glucose. *Diabetes Care*, S62-S66.

3. ADA (2004) Dietary Carbohydrate (Amount and Type) in the Prevention and Management of Diabetes. A statement by the American Diabetes Association. *Diabetes Care* **27**, 2266-2271.

4. ADA (2006) Diagnosis and classification of diabetes mellitus. *Diabetes Care* **29**, S43-S48.

5. Affenito SG (2007) Breakfast: a missed opportunity. *J Am Diet Assoc* **107**, 565-569.

6. Ainsworth BE, Haskell WL, Leon AS, Jacobs DR, Jr., Montoye HJ, Sallis JF & Paffenbarger RS, Jr. (1993) Compendium of physical activities: classification of energy costs of human physical activities. *Med Sci Sports Exerc* **25**, 71-80.

7. Ainsworth BE, Haskell WL, Whitt MC, *et al.* (2000) Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc* **32**, S498-S504.

8. Alfenas RC & Mattes RD (2005) Influence of glycemic index/load on glycemic response, appetite, and food intake in healthy humans. *Diabetes Care* **28**, 2123-2129.

9. Arnold DH & Doctoroff GL (2003) The early education of socioeconomically disadvantaged children. *Annu Rev Psychol* **54**, 517-545.

10. Arvidsson-Lenner R, Asp NG, Axelsen M, *et al.* (2004) Glycaemic index. *Scand J Food Nutr* **48**, 84-94.

11. Awad N, Gagnon M, Desrochers A, Tsiakas M & Messier C (2002) Impact of peripheral glucoregulation on memory. *Behav Neurosci* **116**, 691-702.

12. Balleine BW & Killcross S (2006) Parallel incentive processing: an integrated view of amygdala function. *Trends Neurosci* **29**, 272-279.

13. Barclay A, Brand-Miller J & Wolever TM (2005) Glycemic Index, Glycemic Load, and Glycemic Response Are Not the Same. *Diabetes Care* **28**, 1839.

14. Benton D (2001) The impact of the supply of glucose to the brain on mood and memory. *Nutr Rev* **59**, S20-S21.

15. Benton D (2002) Carbohydrate ingestion, blood glucose and mood. *Neurosci Biobehav Rev* **26**, 293-308.

16. Benton D, Brett V & Brain P (1987) Glucose improves attention and reaction to frustration in children. *Biol Psychol* **24**, 100-105.

17. Benton D & Jarvis M (2007) The role of breakfast and a mid-morning snack on the ability of children to concentrate at school. *Physiol Behav* **90**, 382-385.

18. Benton D, Maconie A & Williams C (2007) The influence of the glycaemic load of breakfast on the behaviour of children in school. *Physiol Behav* **92**, 717-724.

19. Benton D & Nabb S (2003) Carbohydrate, memory, and mood. *Nutr Rev* **61**, S61-S67.

20. Benton D & Owens D (1993) Blood glucose and human memory. *Psychopharmacology (Berl)* **113**, 83-88.

21. Benton D, Owens D & Perales CG (1994) Blood glucose influences memory and attention in young adults. *Neuropsychobiology* **32**, 595-607.

22. Benton D & Parker PY (1998) Breakfast, blood glucose, and cognition. *Am J Clin Nutr* **67**, S772-S778.

23. Benton D, Ruffin MP, Lassel T, Nabb S, Messaoudi M, Vinoy S, Desor D & Lang V (2003) The delivery rate of dietary carbohydrates affects cognitive performance in both rats and humans. *Psychopharmacology (Berl)* **166**, 86-90.

24. Blatter K & Cajochen C (2007) Circadian rhythms in cognitive performance: methodological constraints, protocols, theoretical underpinnings. *Physiol Behav* **90**, 196-208.

25. Bradley RH & Corwyn RF (2002) Socioeconomic status and child development. *Annu Rev Psychol* **53**, 371-399.

26. Brand-Miller J & Holt S (2004) Testing the glycaemic index of foods: in vivo, not in vitro. *Eur J Clin Nutr* **58**, 700-701.

27. Brand-Miller JC, Thomas M, Swan V, Ahmad ZI, Petocz P & Colagiuri S (2003) Physiological validation of the concept of glycemic load in lean young adults. *J Nutr* **133**, 2728-2732.

28. Briony Thomas (2001) *Manual of Dietetic Practice*, Briony Thomas in conjuction with the British Dietetic Association ed.: Blackwell Science.

29. Brouns F, Bjorck I, Frayn KN, Gibbs AL, Lang V, Slama G & Wolever TMS (2005) Glycaemic Index methodology. *Nutrition Research Reviews* **18**, 145-171.

30. Brown AM & Ransom BR (2007) Astrocyte glycogen and brain energy metabolism. *Glia* **55**, 1263-1271.

31. Bryan J, Osendarp S, Hughes D, Calvaresi E, Baghurst K & van Klinken JW (2004) Nutrients for cognitive development in school-aged children. *Nutr Rev* **62**, 295-306.

32. Buchanan TW & Lovallo WR (2001) Enhanced memory for emotional material following stress-level cortisol treatment in humans. *Psychoneuroendocrinology* **26**, 307-317.

33. Buyken AE, Dettmann W, Kersting M & Kroke A (2005) Glycaemic index and glycaemic load in the diet of healthy schoolchildren: trends from 1990 to 2002,

contribution of different carbohydrate sources and relationships to dietary quality. *British Journal of Nutrition 94(5):796-803.*

34. Buyken AE, Trauner K, Gunther AL, Kroke A & Remer T (2007) Breakfast glycemic index affects subsequent daily energy intake in free-living healthy children. *Am J Clin Nutr* **86**, 980-987.

35. Campbell JE, Glowczewski T & Wolever TMS (2003) Controlling subjects' prior diet and activities does not reduce within-subject variation of postprandial glycemic responses to foods. *Nutrition Research* **23**, 621-629.

36. Capaldi I, V, Handwerger K, Richardson E & Stroud LR (2005) Associations between sleep and cortisol responses to stress in children and adolescents: a pilot study. *Behav Sleep Med* **3**, 177-192.

37. Carr M & Davis H (2001) Gender Differences in Arithmetic Strategy Use: A Function of Skill and Preference. *Contemp Educ Psychol* **26**, 330-347.

38. Carrol JB (1993) *Human Cognitive Abilities; A survey of factor-analytic studies*: Cambridge University Press.

39. CDC (1997) *Capillary blood sampling protocol.*

40. Champ M, Langkilde AM, Brouns F, Kettlitz B & Bail-Collet YL (2003) Advances in dietary fibre characterisation. 2. Consumption, chemistry, physiology and measurement of resistant starch; implications for health and food labelling. *Nutrition Research Reviews* **16**, 143-161.

41. Chandler AM, Walker SP, Connolly K & Grantham-McGregor SM (1995) School breakfast improves verbal fluency in undernourished Jamaican children. *J Nutr* **125**, 894-900.

42. Chew I, Brand J, Thorburn A & Truswell A (1988) Application of glycemic index to mixed meals. *Am J Clin Nutr* **47**, 53-56.

43. Cole TJ, Flegal KM, Nicholls D & Jackson AA (2007) Body mass index cut offs to define thinness in children and adolescents: international survey. *BMJ* **335**, 194.

44. Collier G, McLean A & ODea K (1984) Effect of co-ingestion of fat on the metabolic responses to slowly and rapidly absorbed carbohydrates. *Diabetologia* **26**, 50-54.

45. Collier G, Wolever TM, Wong G & Josse R (1986) Prediction of glycemic response to mixed meals in nonisulin-dependent diabetic subjects. *Am J Clin Nutr* **44**, 349-352.

46. Conners CK & Blouin AG (1982) Nutritional effects on behavior of children. *J Psychiatr Res* **17**, 193-201.

47. Craft S, Murphy C & Wemstrom J (1994) Glucose effects on complex memory and nonmemory tasks: The influence of age, sex, and glucoregulatory response. *Phychobiology* **22**, 95-105.

48. Cromer BA, Tarnowski KJ, Stein AM, Harton P & Thornton DJ (1990) The school breakfast program and cognition in adolescents. *J Dev Behav Pediatr* **11**, 295-300.

49. CWT & NHF (2005) *Eating well at School: Nutritional and Practical guidelines*: The Caroline Walker Trust.

50. D'Anci KE, Constant F & Rosenberg IH (2006) Hydration and cognitive function in children. *Nutrition Reviews* **64**, 457-464.

51. D'Orazio P, Burnett RW, Fogh-Andersen N, *et al.* (2005) Approved IFCC recommendation on reporting results for blood glucose (abbreviated). *Clin Chem* **51**, 1573-1576.

52. Davydov DM, Shapiro D, Goldstein IB & Chicz-DeMet A (2005) Moods in everyday situations: effects of menstrual cycle, work, and stress hormones. *J Psychosom Res* **58**, 343-349.

53. de la Hunty A & Ashwell M (2007) Are people who regularly eat breakfast cereals slimmer than those who don't? A systematic review of the evidence. *Nutrition Bulletin* **32**, 118-128.

54. DH (1991) *Department of Health; Report on Health and Social Subjects No.41. Dietary Reference Values for Food Energy and Nutrients for the United Kingdom*: HMSO, London.

55. Dickie NH & Bender AE (1982) Breakfast and performance in school children. *Br J Nutr* **48**, 483-496.

56. Dolan RJ (2002) Emotion, cognition, and behavior. *Science* **298**, 1191-1194.

57. Donohoe RT & Benton D (1999a) Cognitive functioning is susceptible to the level of blood glucose. *Psychopharmacology (Berl)* **145**, 378-385.

58. Donohoe RT & Benton D (1999b) Declining blood glucose levels after a cognitively demanding task predict subsequent memory. *Nutr Neurosci* **2**, 413-24.

59. Donohoe RT & Benton D (2000) Glucose tolerance predicts performance on tests of memory and cognition. *Physiol Behav* **71**, 395-401.

60. Drever J. (1964) *A Dictionary of Psychology*, Revised ed.: Harmondworth: Penguin Books.

61. Dye L, Lluch A & Blundell JE (2000) Macronutrients and mental performance. *Nutrition* **16**, 1021-1034.

62. Ebbeling CB, Leidig MM, Sinclair KB, Seger-Shippee LG, Feldman HA & Ludwig DS (2005) Effects of an ad libitum low-glycemic load diet on cardiovascular disease risk factors in obese young adults. *Am J Clin Nutr* **81**, 976-982.

63. Elliot C, Smith P & McCulloch K (1996) *British Ability Scales (BAS II)*, Second ed. Windsor, Berkshire.

64. Evans ML, Pernet A, Lomas J, Jones J & Amiel SA (2000) Delay in onset of awareness of acute hypoglycemia and of restoration of cognitive performance during recovery. *Diabetes Care* **23**, 893-897.

65. Fairclough SH & Houston K (2004) A metabolic measure of mental effort. *Biol Psychol* **66**, 177-190.

66. Fanjiang G & Kleinman RE (2007) Nutrition and performance in children. *Curr Opin Clin Nutr Metab Care* **10**, 342-347.

67. FAO/WHO (1998) *Carbohydrates in Human Nutrition (FAO Food and Nutrition Paper - 66).*

68. Fernstrom JD (1983) Role of precursor availability in control of monoamine biosynthesis in brain. *Physiol Rev* **63**, 484-546.

69. Fernstrom MH & Fernstrom JD (1995) Brain tryptophan concentrations and serotonin synthesis remain responsive to food consumption after the ingestion of sequential meals. *Am J Clin Nutr* **61**, 312-319.

70. Finch S, Doyle W, Lowe S, Bates CJ, Prentice A, Smithers G & Clarke PC (1998) *National Diet and Nutrition Survey: people aged 65 years and over*: London: TSO.

71. Fischer K, Colombani PC, Langhans W & Wenk C (2002) Carbohydrate to protein ratio in food and cognitive performance in the morning. *Physiol Behav* **75**, 411-423.

72. Flint A, Moller BK, Raben A, Pedersen D, Tetens I, Holst JJ & Astrup A (2004) The use of glycaemic index tables to predict glycaemic index of composite breakfast meals. *Br J Nutr* **91**, 979-989.

73. Flint RW, Jr. & Turek C (2003) Glucose effects on a continuous performance test of attention in adults. *Behav Brain Res* **142**, 217-228.

74. Foster JK, Lidder PG & Sunram SI (1998) Glucose and memory: fractionation of enhancement effects? *Psychopharmacology (Berl)* **137**, 259-270.

75. Foster-Powell K, Holt S & Brand-Miller J (2002) International Table of Glycemic index and Glycemic load values. *Am J Clin Nutr* **76**, 5-56.

76. FSA (2002) *McCance and Widdowson's the Composition of Foods, 6th summary edition.*

77. Galgani J, Aguirre C & Diaz E (2006) Acute effect of meal glycemic index and glycemic load on blood glucose and insulin responses in humans. *Nutrition Journal 5:22.*

78. Geary DC (2006) Sex differences in social behavior and cognition: Utility of sexual selection for hypothesis generation. *Hormones & Behavior* **49**, 273-275.

79. Genazzani AR, Facchinetti F, Pintor C, Puggioni R, Parrini D, Petraglia F, Bagnoli F & Corda R (1983) Proopiocortin-related peptide plasma levels throughout prepuberty and puberty. *J Clin Endocrinol Metab* **57**, 56-61.

80. Gibson & Green MW (2002) Nutritional influences on cognitive function: mechanisms of susceptibility. *Nutrition Research Reviews* **15**, 169-206.

81. Gibson EL (2007) Carbohydrates and mental function: feeding or impeding the brain? *Nutrition Bulletin* **32**, 71-83.

82. Gogtay N, Giedd JN, Lusk L, *et al.* (2004) Dynamic mapping of human cortical development during childhood through early adulthood. *Proc Natl Acad Sci U S A* **101**, 8174-8179.

83. Gonzalez-Bono E, Rohleder N, Hellhammer DH, Salvador A & Kirschbaum C (2002) Glucose but Not Protein or Fat Load Amplifies the Cortisol Response to Psychosocial Stress. *Hormones and Behavior* **41**, 328-333.

84. Gordon N (2003) Iron deficiency and the intellect. *Brain and Development* **25**, 3-8.

85. Government Statistical Service (1990) *Standard Occupational Classification; Volumes 1, 2 & 3.*

86. Government Statistical Service (2000) *Standard Occupational Classification; Volumes 1 & 2.*

87. Granfeldt Y, Wu X & Bjorck I (2006) Determination of glycaemic index; some methodological aspects related to the analysis of carbohydrate load and characteristics of the previous evening meal. *Eur J Clin Nutr* **60**, 104-112.

88. Green MW, Elliman NA & Rogers PJ (1997) The effects of food deprivation and incentive motivation on blood glucose levels and cognitive function. *Psychopharmacology (Berl)* **134**, 88-94.

89. Green MW & Rogers PJ (1995) Impaired cognitive functioning in dieters during spontaneous dieting. *Psychol Med* **25**, 1003-1010.

90. Green MW, Taylor MA, Elliman NA & Rhodes O (2001) Placebo expectancy effects in the relationship between glucose and cognition. *Br J Nutr* **86**, 173-179.

91. Gregory JR, Collins DL, Davies PSW, Hughes JM & Clarke PC (1995) *National Diet and Nutrition Survey: children aged 1½ to 4½ years*: London: HMSO.

92. Gregory JR, Foster JK, Tyler H & Wiseman H (1990) *The Dietary and Nutritional Survey of British Adults*: London: HMSO.

93. Gregory JR, Lowe S, Bates CJ, Prentice A, Jackson LV, Smithers G, Wenlock R & Farron M (2000) *National Diet and Nutrition Survey: young people aged 4 to 18 years*: London: TSO.

94. Groome D, Dewart E, Esgate A, Gurney K & Towell N (1999) *An Introduction to Cognitive Psychology: Processes and Disorders*: Routledge.

95. Gunstad J, Spitznagel MB, Paul RH, Cohen RA, Kohn M, Luyster FS, Clark R, Williams LM & Gordon E (2008) Body mass index and neuropsychological function in healthy children and adolescents. *Appetite* **50**, 246-251.

96. Halpern D (1992) *Sex differences in cognitive abilities*. New Jersey: Lawrence Erlbaum.

97. Halterman JS, Kaczorowski JM, Aligne CA, Auinger P & Szilagyi PG (2001) Iron deficiency and cognitive achievement among school-aged children and adolescents in the United States. *Pediatrics* **107**, 1381-1386.

98. Hanrahan K, McCarthy AM, Kleiber C, Lutgendorf S & Tsalikian E (2006) Strategies for salivary cortisol collection and analysis in research with children. *Appl Nurs Res* **19**, 95-101.

99. Hayman M (1942) Two minute clinical tests for measurement of intellectual impairment in psychiatric disorders. *Arch Neurol Psychatry* **47**, 454-464.

100. Heatherley SV, Hayward RC, Seers HE & Rogers PJ (2005) Cognitive and psychomotor performance, mood, and pressor effects of caffeine after 4, 6 and 8 h caffeine abstinence. *Psychopharmacology (Berl)* **178**, 461-470.

101. Henderson L, Gregory JR & Swan J (2003) *National Diet and Nutrition Survey: adults aged 19 to 64 years*: London: HMSO.

102. Henry CJ, Lightowler HJ, Strik CM, Renton H & Hails S (2005) Glycaemic index and glycaemic load values of commercially available products in the UK. *Br J Nutr* **94**, 922-930.

103. Hertz L, Peng L & Dienel GA (2007) Energy metabolism in astrocytes: high rate of oxidative metabolism and spatiotemporal dependence on glycolysis/glycogenolysis. *J Cereb Blood Flow Metab* **27**, 219-249.

104. Hoge RD & Pike GB (2001) Oxidative metabolism and the detection of neuronal activation via imaging. *J Chem Neuroanat* **22**, 43-52.

105. Holt SH, Delargy HJ, Lawton CL & Blundell JE (1999) The effects of high-carbohydrate vs high-fat breakfasts on feelings of fullness and alertness, and subsequent food intake. *Int J Food Sci Nutr* **50**, 13-28.

106. Hoyland A, Lawton CL & Dye L (2008) Acute effects of macronutrient manipulations on cognitive test performance in healthy young adults: A systematic research review. *Neuroscience & Biobehavioral Reviews* **32**, 72-85.

107. ICSH (1978) International Committee for Standardization in Haematology. Recommendations for reference method for haemoglobinometry in human blood (ICSH standard EP *6/2: 1977) and* specifications for international haemiglobincyanide reference preparation (ICSH standard *EP 6/3: 1977). J Clin Pathol* **31**, 139-143.

108. Ingwersen J, Defeyter MA, Kennedy DO, Wesnes KA & Scholey AB (2007) A low glycaemic index breakfast cereal preferentially prevents children's cognitive performance from declining throughout the morning. *Appetite* **49**, 240-244.

109. Jacobsen LK, Krystal JH, Mencl WE, Westerveld M, Frost SJ & Pugh KR (2005) Effects of smoking and smoking abstinence on cognition in adolescent tobacco smokers. *Biol Psychiatry* **57**, 56-66.

110. James JE & Rogers PJ (2005) Effects of caffeine on performance and mood: withdrawal reversal is the most plausible explanation. *Psychopharmacology (Berl)*, 1-8.

111. Jenkins DJ, Wolever TM, Taylor R, Barker H, Fielden H, Baldwin J, Bowling A, Newman H, Jenkins A & Goff D (1981) Glycemic Index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr* **34**, 362-366.

112. Kanarek R (1997) Psychological effects of snacks and altered meal frequency. *Br J Nutr* **77**, S105-S118.

113. Kaplan RJ, Greenwood CE, Winocur G & Wolever TM (2000) Cognitive performance is associated with glucose regulation in healthy elderly persons and can be enhanced with glucose and dietary carbohydrates. *Am J Clin Nutr* **72**, 825-836.

114. Kennedy DO & Scholey AB (2000) Glucose administration, heart rate and cognitive performance: effects of increasing mental effort. *Psychopharmacology (Berl)* **149**, 63-71.

115. Kern W, Peters A, Fruehwald-Schultes B, Deininger E, Born J & Fehm HL (2001) Improving influence of insulin on cognitive functions in humans. *Neuroendocrinology* **74**, 270-280.

116. King SL & Hegadoren KM (2002) Stress hormones: how do they measure up? *Biol Res Nurs* **4**, 92-103.

117. Kirschbaum C, Gonzalez BE, Rohleder N, Gessner C, Pirke KM, Salvador A & Hellhammer DH (1997) Effects of fasting and glucose load on free cortisol responses to stress and nicotine. *J Clin Endocrinol Metab* **82**, 1101-1105.

118. Kirschbaum C & Hellhammer DH (1994) Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology* **19**, 313-333.

119. Kirschbaum C, Wolf OT, May M, Wippich W & Hellhammer DH (1996) Stress- and treatment-induced elevations of cortisol levels associated with impaired declarative memory in healthy adults. *Life Sci* **58**, 1475-1483.

120. Kretchmer N, Beard JL & Carlson S (1996) The role of nutrition in the development of normal cognition. *Am J Clin Nutr* **63**, 997S-1001S.

121. Lifescan (1998) *Blood glucose monitoring systems - current technologies.*

122. Liu P, Perry T & Monro JA (2003) Glycaemic glucose equivalent: validation as a predictor of the relative glycaemic effect of foods. *Eur J Clin Nutr* **57**, 1141-1149.

123. Lloyd HM, Rogers PJ, Hedderley DI & Walker AF (1996) Acute effects on mood and cognitive performance of breakfasts differing in fat and carbohydrate content. *Appetite*, 151-164.

124. Lombard CB (2000) What is the role of food in preventing depression and improving mood, performance and cognitive function? *Med J Aust* **173**, S104-S105.

125. Lopez I, de A, I, Perales CG, Heresi E, Castillo M & Colombo M (1993) Breakfast omission and cognitive performance of normal, wasted and stunted schoolchildren. *Eur J Clin Nutr* **47**, 533-542.

126. Lopez-Sobaler AM, Ortega RM, Quintas ME, Navia B & Requejo AM (2003) Relationship between habitual breakfast and intellectual performance (logical reasoning) in well-nourished schoolchildren of Madrid (Spain). *Eur J Clin Nutr* **57**, 49-53.

127. Lorr M & McNair D (1988) *Profile of mood states bi-polar form.* San Diego.

128. Ludwig DS (2002) The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *JAMA* **287**, 2414-2423.

129. Lupien SJ, Gillin CJ & Hauger RL (1999) Working memory is more sensitive than declarative memory to the acute effects of corticosteroids: a dose-response study in humans. *Behav Neurosci* **113**, 420-430.

130. Lupien SJ, Maheu F, Tu M, Fiocco A & Schramek TE (2007) The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition. *Brain Cogn* **65**, 209-237.

131. Lupien SJ, Wilkinson CW, Briere S, Menard C, Ng Ying Kin NM & Nair NP (2002) The modulatory effects of corticosteroids on cognition: studies in young human populations. *Psychoneuroendocrinology* **27**, 401-416.

132. Macht M (1996) Effects of high- and low-energy meals on hunger, physiological processes and reactions to emotional stress. *Appetite* **26**, 71-88.

133. Mahoney CR, Taylor HA, Kanarek RB & Samuel P (2005) Effect of breakfast composition on cognitive processes in elementary school children. *Physiol Behav* **85**, 635-645.

134. Manly T, Anderson V, Nimmo-Smith I, Turner A, Watson P & Robertson IH (2001) The differential assessment of children's attention: the Test of Everyday Attention for Children (TEA-Ch), normative sample and ADHD performance. *J Child Psychol Psychiatry* **42**, 1065-1081.

135. Manning C, Hall J & Gold P (1990) Glucose effects on memory and other neuropsychological tests in elderly humans. *Psychol Sci* **1**, 307-311.

136. Markus CR, Panhuysen G, Jonkman LM & Bachman M (1999) Carbohydrate intake improves cognitive performance of stress-prone individuals under controllable laboratory stress. *Br J Nutr* **82**, 457-467.

137. Markus CR, Panhuysen G, Tuiten A, Koppeschaar H, Fekkes D & Peters ML (1998) Does carbohydrate-rich, protein-poor food prevent a deterioration of mood and cognitive performance of stress-prone subjects when subjected to a stressful task? *Appetite* **31**, 49-65.

138. Matthys C, De Henauw S, Bellemans M, De Maeyer M & De Backer G (2007) Breakfast habits affect overall nutrient profiles in adolescents. *Public Health Nutr* **10**, 413-421.

139. McCann JC & Ames BN (2007) An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. *Am J Clin Nutr* **85**, 931-945.

140. McNay EC, Fries TM & Gold PE (2000) Decreases in rat extracellular hippocampal glucose concentration associated with cognitive demand during a spatial task. *Proc Natl Acad Sci U S A* **97**, 2881-2885.

141. McNay EC & Gold PE (1997) Microdialysis measurement of brain extracellular glucose concentrations: basal levels and changes during behavioral testing in awake freely moving rats. *Soc Neurosci Abstr*, 823-816.

142. McNay EC & Gold PE (1999) Extracellular glucose concentrations in the rat hippocampus measured by zero-net flux: effects of microdialysis flow rate, strain and age. *Neurochemistry* **72**, 785-790.

143. McNay EC, McCarty RC & Gold PE (2001) Fluctuations in brain glucose concentration during behavioral testing: dissociations between brain areas and between brain and blood. *Neurobiol Learn Mem* **75**, 325-337.

144. MDA (1996) *Safety Notice* no. 9616.

145. MDA (2004) *TheraSense FreeStyle blood glucose meter; evaluation report 02049.*

146. Meikle A, Riby LM & Stollery B (2005) Memory processing and the glucose facilitation effect: The effects of stimulus difficulty and memory load. *Nutr Neurosci* **8**, 227-232.

147. Melanson EL, Jr. & Freedson PS (1996) Physical activity assessment: a review of methods. *Crit Rev Food Sci Nutr* **36**, 385-396.

148. Messier C (2004) Glucose improvement of memory: a review. *Eur J Pharmacol* **490**, 33-57.

149. Messier C, Desrochers A & Gagnon M (1999) Effect of glucose, glucose regulation, and word imagery value on human memory. *Behav Neurosci* **113**, 431-438.

150. Messier C, Durkin T, Mrabet O & Destrade C (1990) Memory-improving action of glucose: indirect evidence for a facilitation of hippocampal acetylcholine synthesis. *Behav Brain Res* **39**, 135-143.

151. Messier C, Gagnon M & Knott V (1997) Effect of Glucose and Peripheral Glucose Regulation on Memory in the Elderly. *Neurobiol Aging* **18**, 297-304.

152. Messier C, Tsiakas M, Gagnon M, Desrochers A & Awad N (2003) Effect of age and glucoregulation on cognitive performance. *Neurobiol Aging* **24**, 985-1003.

153. Michaud C, Musse N, Nicolas JP & Mejean L (1991) Effects of breakfast-size on short-term memory, concentration, mood and blood glucose. *Journal of Adolescent Health* **12**, 53-57.

154. Millan-Price J & Brand-Miller J (2006) Low-glycaemic index diets and body weight regulation. *Int J Obes* **30**, S40-S46.

155. Monro JA & Williams M (2000) Concurrent management of postprandial glycaemia and nutrient intake using glycaemic glucose equivalents, food composition data and computer-assisted meal design. *Asia Pac J Clin Nutr* **9**, 67-73.

156. Moshfegh AJ, Rhodes DG, Baer DJ, *et al.* (2008) The US Department of Agriculture Automated Multiple-Pass Method reduces bias in the collection of energy intakes. *Am J Clin Nutr* **88**, 324-332.

157. Nabb S & Benton D (2006) The influence on cognition of the interaction between the macro-nutrient content of breakfast and glucose tolerance. *Physiol Behav* **87**, 16-23.

158. NDNS (2000) *National Diet and Nutrition Survey: young people aged 4 to 18 years.*

159. Neese JW, Duncan P, Bayse D, Robinson M, Cooper T & Stewart C (1976) *Center for Disease Control: Development and Evaluation of a Hexokinase/ Glucose-6-Phosphate Dehydrogenase Procedure for Use as National Glucose Reference Method* no. DHWE Pub.No.(CDC) 77-8330. Atlanta, GA.

160. Nelson M (1996) Anaemia in adolescent girls: effects on cognitive function and activity. *Proc Nutr Soc* **55**, 359-367.

161. Nelson M, Ash R, Mulvihill C, Peters T & Rogers PJ (2001) Iron status, diet and cognitive function in British adolescent girls. *Proc Nutr Soc* **60**, 59A.

162. Nelson M, Atkins M & Meyer J (1997) *Food Portion Sizes, a user's guide to photographic atlas*: MAFF.

163. Nelson M, Atkinson M & Darbyshire S (1996) Food photography II: use of food photographs for estimating portion size and the nutrient content of meals. *Br J Nutr* **76**, 31-49.

164. Nelson M, Bakaliou F & Trivedi A (1994) Iron-deficiency anaemia and physical performance in adolescent girls from different ethnic backgrounds. *Br J Nutr* **72**, 427-433.

165. Nelson M, Bradbury J, Poulter J, McGee A, Msebele S & Jarvis L (2004) *School Meals in Secondary Schools in England* no. FSA Research Report 557.

166. Nelson M, Erens B, Bates B, Church S & Boshier T (2007) *Low Income Diet and Nutrition Survey*: London: TSO.

167. Nelson M, Nicholas J, Suleiman S, Davies O, Prior G, Hall L, Wreford S & Poulter J (2006) *School Meals in Primary Schools in England* no. FSA Research Report 753.

168. NHS (2004a) *Blood glucose monitoring guidelines for practice-NHS.*

169. NHS (2004b) *Clinical guidelines for capillary blood glucose monitoring* no. IT.CBGM.31.01.01.3.

170. NHS (2004c) *Guidelines for adult venepuncture* no. IT.VN.21.12.04.

171. NHS PASA (2005) *Report 05072; Roche Diagnostics Accu-Chek Aviva blood glucose meter.*

172. Nicklas TA, mory-Luce D, Yang SJ, Baranowski T, Zakeri I & Berenson G (2004) Children's food consumption patterns have changed over two decades (1973-1994): The Bogalusa heart study. *J Am Diet Assoc* **104**, 1127-1140.

173. Nuttal F, Mooradian A, Gannon M, Billington C & Krezowski P (1984) Effect of protein ingestion on the glucose and insulin response to a standadized oral glucose load. *Diabetes Care* **7**, 465-470.

174. Owens DS & Benton D (1994) The Impact of raising blood glucose on reaction times. *Neuropsychobiology.*

175. Owens DS, Parker PY & Benton D (1997) Blood glucose and subjective energy following cognitive demand. *Physiol Behav* **62**, 471-478.

176. Parsons M & Gold P (1992) Glucose enhancement of memory in elderly humans: An incerted U-dose response curve. *Neurobiological Aging* **13**, 401-404.

177. Pasman WJ, Blokdijk VM, Bertina FM, Hopman WP & Hendriks HF (2003) Effect of two breakfasts, different in carbohydrate composition, on hunger and satiety and

mood in healthy men. *International Journal of Obesity & Related Metabolic Disorders* **27**, 663-668.

178. Paulson OB (2002) Blood-brain barrier, brain metabolism and cerebral blood flow. *Eur Neuropsychopharmacol* **12**, 495-501.

179. Peters A, Schweiger U, Pellerin L, Hubold C, Oltmanns KM, Conrad M, Schultes B, Born J & Fehm HL (2004) The selfish brain: competition for energy resources. *Neurosci Biobehav Rev* **28**, 143-180.

180. Pi-Sunyer F (2002) Glycemic Index and Disease. *Am J Clin Nutr* **76**, 290S-298S.

181. Pi-Sunyer X (2005) Do Glycemic Index, Glycemic Load, and Fiber Play a Role in Insulin Sensitivity, Disposition Index, and Type 2 Diabetes? *Diabetes Care* **28**, 2978-2979.

182. Pollitt E, Jacoby E & Cueto S (1996) School breakfast and cognition among nutritionally at-risk children in the Peruvian Andes. *Nutr Rev* **54**, S22-S26.

183. Pollitt E, Leibel RL & Greenfield D (1981) Brief fasting, stress, and cognition in children. *Am J Clin Nutr* **34**, 1526-1533.

184. Pollitt E, Lewis NL, Garza C & Shulman RJ (1982) Fasting and cognitive function. *J Psychiatr Res* **17**, 169-174.

185. Pollitt E & Mathews R (1998) Breakfast and cognition: an integrative summary. *Am J Clin Nutr* **67**, S804-S813.

186. Power C, Jefferis BJ, Manor O & Hertzman C (2006) The influence of birth weight and socioeconomic position on cognitive development: Does the early home and learning environment modify their effects? *J Pediatr* **148**, 54-61.

187. Rampersaud GC, Pereira MA, Girard BL, Adams J & Metzl JD (2005) Breakfast habits, nutritional status, body weight, and academic performance in children and adolescents. *J Am Diet Assoc* **105**, 743-760.

188. Reid M & Hammersley R (1999) The effects of carbohydrates on arousal. *Nutrition Research Reviews* **12**, 3-23.

189. Reid M (2007) The effects of nutrients on mood and behaviour. *Nutrition Bulletin* **32**, 1-3.

190. Rennie KL & Wareham NJ (1998) The validation of physical activity instruments for measuring energy expenditure: problems and pitfalls. *Public Health Nutr* **1**, 265-271.

191. Reynolds RM, Walker BR, Syddall HE, Whorwood CB, Wood PJ & Phillips DI (2001) Elevated plasma cortisol in glucose-intolerant men: differences in responses to glucose and habituation to venepuncture. *J Clin Endocrinol Metab* **86**, 1149-1153.

192. Riby LM (2004) The Impact of Age and Task Domain on Cognitive Performance: A Meta-Analytic Review of the Glucose Facilitation Effect. *Brain Impairment* **5**, 145-65.

193. Riby LM, McMurtrie H, Smallwood J, Ballantyne C, Meikle A & Smith E (2006) The facilitative effects of glucose ingestion on memory retrieval in younger and older adults: is task difficulty or task domain critical? *Br J Nutr* **95**, 414-420.

194. Rocha FT, Rocha AF, Massad E & Menezes R (2005) Brain mappings of the arithmetic processing in children and adults. *Brain Res Cogn Brain Res* **22**, 359-372.

195. Rogers PJ (2007) Caffeine, mood and mental performance in everyday life. *Nutrition Bulletin* **32**, 84-89.

196. Rogers PJ, Edwards S, Green MW & Jas P (1992) Nutritional influences on mood and cognitive performance: the menstrual cycle, caffeine and dieting. *Proc Nutr Soc* **51**, 343-351.

197. Rogers PJ, Richardson NJ & Elliman NA (1995) Overnight caffeine abstinence and negative reinforcement of preference for caffeine-containing drinks. *Psychopharmacology (Berl)* **120**, 457-462.

198. Rohleder N & Kirschbaum C (2007) Effects of nutrition on neuro-endocrine stress responses. *Curr Opin Clin Nutr Metab Care* **10**, 504-510.

199. Rolls ET & Kesner RP (2006) A computational theory of hippocampal function, and empirical tests of the theory. *Prog Neurobiol* **79**, 1-48.

200. Rosmond R & Bjorntorp P (2000) Occupational status, cortisol secretory pattern, and visceral obesity in middle-aged men. *Obes Res* **8**, 445-450.

201. Sachdev H, Gera T & Nestel P (2005) Effect of iron supplementation on mental and motor development in children: systematic review of randomised controlled trials. *Public Health Nutr* **8**, 117-132.

202. Salmeron J, Manson J, Stampfer M, Colditz G, Wing A & Willet W (1997) Dietary Fiber, Glycemic Load, and Risk of Non-insulin-dependent Diabetes Mellitus in Women. *JAMA* **277**, 472-477.

203. Schmitt JA, Benton D & Kallus KW (2005) General methodological considerations for the assessment of nutritional influences on human cognitive functions. *Eur J Nutr* **44**, 459-464.

204. Scholey AB (2001) Fuel for thought. *The phychologist* **14**, 196-201.

205. Scholey AB, Harper S & Kennedy DO (2001) Cognitive demand and blood glucose. *Physiol Behav* **73**, 585-592.

206. SFT (2006a) *A guide to introducing the Government's new food-based standards for all school food other than lunches.*

207. SFT (2006b) *A guide to introducing the Government's new food-based standards for lunch.*

208. Siega-Riz AM, Popkin BM & Carson T (1998) Trends in breakfast consumption for children in the United States from 1965-1991. *Am J Clin Nutr* **67**, S748-S756.

209. Silverthorn DE (2001) *Human Physiology. An Integrated Approach*, Second ed.

210. Simeon DT & Grantham-McGregor S (1989) Effects of missing breakfast on the cognitive functions of school children of differing nutritional status. *Am J Clin Nutr* **49**, 646-653.

211. Slavin J & Green H (2007) Dietary fibre and satiety. *Nutrition Bulletin* **32**, 32-42.

212. Smith A, Kendrick A, Maben A & Salmon J (1994) Effects of breakfast and caffeine on cognitive performance, mood and cardiovascular functioning. *Appetite* **22**, 39-55.

213. Smith AP (2002) Stress, breakfast cereal consumption and cortisol. *Nutr Neurosci* **5**, 141-144.

214. Smith AP, Clark R & Gallagher J (1999) Breakfast cereal and caffeinated coffee: effects on working memory, attention, mood, and cardiovascular function. *Physiol Behav* **67**, 9-17.

215. Sodexho (2005) *The Sodexho School Meals and Lifestyle Survey 2005.*

216. Spear LP (2000) The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev* **24**, 417-463.

217. Stevenson J (2006) Dietary influences on cognitive development and behaviour in children. *Proc Nutr Soc* **65**, 361-365.

218. Stroop JR (1935) The basis of Ligon's theory. *Am J Psychol* **47**, 499-504.

219. Stroop JR (1938) Factors affecting speed in serial verbal reactions. *Psychological Monographs* **50**, 38-48.

220. Stuss DT & Levine B (2002) Adult clinical neuropsychology: lessons from studies of the frontal lobes. *Annu Rev Psychol* **53**, 401-433.

221. Sunram-Lea SI, Foster JK, Durlach P & Perez C (2001) Glucose facilitation of cognitive performance in healthy young adults: examination of the influence of fast-duration, time of day and pre-consumption plasma glucose levels. *Psychopharmacology (Berl)* **157**, 46-54.

222. Sunram-Lea SI, Foster JK, Durlach P & Perez C (2002) Investigation into the significance of task difficulty and divided allocation of resources on the glucose memory facilitation effect. *Psychopharmacology (Berl)* **160**, 387-397.

223. Tanner JM, Whitehouse RH & Cameron N (1983) *Assessment of Skeletal Maturity and Prediction of Adult Height*, 2nd ed. London, UK: Academic press.

224. Taylor LA & Rachman SJ (1988) The effects of blood sugar level changes on cognitive function, affective state, and somatic symptoms. *J Behav Med* **11**, 279-291.

225. Turner AP, Chen B & Piletsky SA (1999) In vitro diagnostics in diabetes: meeting the challenge. *Clin Chem* **45**, 1596-1601.

226. Tuttle WW, Daum K, Larsen R, Salzano J & Roloff L (1954) Effect on school boys of omitting breakfast; physiologic responses, attitudes, and scholastic attainments. *J Am Diet Assoc* **30**, 674-677.

227. Tuttle WW, Daum K, Myers L & Martin C (1950) Effect of omitting breakfast on the physiologic response of men. *J Am Diet Assoc* **26**, 332-335.

228. Tuttle WW, Daum K, Roberts H & Randal B (1953) Physiologic response to size and content of breakfast by men over 60. *J Am Diet Assoc* **29**, 34-40.

229. Vaisman N, Voet H, Akivis A & Vakil E (1996) Effect of breakfast timing on the cognitive functions of elementary school students. *Arch Pediatr Adolesc Med* **150**, 1089-1092.

230. Virgin CE, Jr., Ha TP, Packan DR, Tombaugh GC, Yang SH, Horner HC & Sapolsky RM (1991) Glucocorticoids inhibit glucose transport and glutamate uptake in hippocampal astrocytes: implications for glucocorticoid neurotoxicity. *J Neurochem* **57**, 1422-1428.

231. von Schenck H, Falkensson M & Lundberg B (1986) Evaluation of "HemoCue," a new device for determining hemoglobin. *Clin Chem* **32**, 526-529.

232. Waber DP, De MC, Forbes PW, Almli CR, Botteron KN, Leonard G, Milovan D, Paus T & Rumsey J (2007) The NIH MRI study of normal brain development: performance of a population based sample of healthy children aged 6 to 18 years on a neuropsychological battery. *J Int Neuropsychol Soc* **13**, 729-746.

233. Wachs TD (1995) Relation of mild-to-moderate malnutrition to human development: correlational studies. *J Nutr* **125**, S2245-S2254.

234. Wechsler D (1949) *The Wechsler Intelligence Scales for Children: Manual*: Psychological Corporation.

235. Wesnes KA, Pincock C, Richardson D, Helm G & Hails S (2003) Breakfast reduces declines in attention and memory over the morning in schoolchildren. *Appetite* **41**, 329-331.

236. Westenhoefer J, Bellisle F, Blundell JE, de VJ, Edwards D, Kallus W, Milon H, Pannemans D, Tuijtelaars S & Tuorila H (2004) PASSCLAIM--mental state and performance. *Eur J Nutr* **43**, II85-II117.

237. Wheeler ML (2003) Nutrient database for the 2003 exchange lists for meal planning. *J Am Diet Assoc* **103**, 894-920.

238. White AM & Swartzwelder HS (2004) Hippocampal function during adolescence: a unique target of ethanol effects. *Ann N Y Acad Sci* **1021**, 206-220.

239. WHO (1999) *Definition, Diagnosis, and Classification of Diabetes Mellitus and its Complications*. Geneva.

240. WHO (2000) *Obesity: preventing and managing the global epidemic. Report of a WHO Consultation.* Geneva, World Health Organization.

241. WHO (2001) *Iron deficiency anaemia: assessment, prevention and control. A guide for programme managers*. Geneva, Word Health Organization.

242. WHO (2007) *Development of a WHO growth reference for school-aged children and adolescents* no. 85.

243. Wiesli P, Schmid C, Kerwer O, Nigg-Koch C, Klaghofer R, Seifert B, Spinas GA & Schwegler K (2005) Acute psychological stress affects glucose concentrations in

patients with type 1 diabetes following food intake but not in the fasting state. *Diabetes Care* **28**, 1910-1915.

244. Wolever TM (2004) Effect of blood sampling schedule and method of calculating the area under the curve on validity and precision of glycaemic index values. *Br J Nutr* **91**, 295-301.

245. Wolever TM & Bolognesi C (1996a) Source and Amount of Carbohydrate Affect Postprandial Glucose and Insulin in Normal Subjects. *J Nutr* **126**, 2798-2806.

246. Wolever TM & Bolognesi C (1996b) Prediction of glucose and insulin responses of normal subjects after consuming mixed meals varying in energy, protein, fat, carbohydrate and glycemic index. *J Nutr* **126**, 2807-2812.

247. Wolever TM & Jenkins DJ (1986) The use of the glycemic index in predicting the blood glucose response to mixed meals. *Am J Clin Nutr* **43**, 167-172.

248. Wolever TM, Jenkins DJ, Ocana AM, Rao VA & Collier GR (1988) Second-meal effect: low-glycemic-index foods eaten at dinner improve subsequent breakfast glycemic response. *Am J Clin Nutr* **48**, 1041-1047.

249. Wolever TM, Nuttall FQ, Lee R, Wong GS, Josse RG, Csima A & Jenkins DJ (1985) Prediction of the relative blood glucose response of mixed meals using the white bread glycemic index. *Diabetes Care* **8**, 418-428.

250. Wolever TM, Vorster HH, Bjorck I, *et al.* (2003) Determination of the glycaemic index of foods: interlaboratory study. *Eur J Clin Nutr* **57**, 475-482.

251. Wolever TM, Yang M, Zeng XY, Atkinson F & Brand-Miller JC (2006) Food glycemic index, as given in glycemic index tables, is a significant determinant of glycemic responses elicited by composite breakfast meals. *Am J Clin Nutr* **83**, 1306-1312.

252. Wyon D, Abrahamson L, Jartelius M & Fletcher RJ (1997) An experimental study of the effects of energy intake at breakfast on the test performance of 10-year-old children in school. *Int J Food Sci Nutr* **48**, 5-12.

253. Yurgelun-Todd D (2007) Emotional and cognitive changes during adolescence. *Curr Opin Neurobiol* **17**, 251-257.

254. Zerfas AJ (1985) *Checking continuous measures: Manual for anthropometry*. Division of Epidemiology, School of Public Health, University of California, Los Angeles.

255. Zhao WQ, Chen H, Quon MJ & Alkon DL (2004) Insulin and the insulin receptor in experimental models of learning and memory. *Eur J Pharmacol* **490**, 71-81.